BULLETIN
OF THE
TORREY BOTANICAL CLUB

VOLUME 68 FEBRUARY, 1941 NUMBER 2

STUDIES IN THE WORONINACEAE—II. THE CYTOLOGY OF OLPIDIOPSIS ACHLYAE SP. NOV. (AD INT.)

D. A. McLARTY

(WITH EIGHTY-ONE FIGURES)

INTRODUCTION

Sexuality in the Chytridiales was first demonstrated by Cornu (1872) in the formation of *Olpidiopsis* resting spores. This fusion of thalli of unequal size has stimulated the interest of many mycologists and has led to the view that members of this genus may be ancestral to the Saprolegniales. Few critical observations, however, have been made on resting spore formation in any of these species. Life cycle studies and observations on plasmogamy in the formation of resting spores have been made by Reinsch (1878), Fischer (1880, 1882), Dangeard (1890), Maurizio (1895), Butler (1907), and others for various species of *Olpidiopsis*. The most complete cytological study which has been made on this genus, however, was published by Barrett in 1912. His studies include observations on the penetration of the host and the developmental stages of the zoosporangia and resting spores in three species of *Olpidiopsis*. He has demonstrated that the male and female thalli are multinucleate when plasmogamy occurs and states that the resting spore, at maturity, contains many small nuclei. His illustrations of these stages, however, leave much to be desired. Moreover, he has not demonstrated the fate of the supernumerary male nuclei which may enter the female thallus from several smaller thalli nor has he shown conclusively that karyogamy occurs in the formation of the so-called sexual resting spores. At that time sexuality was looked upon as a well developed, constant feature in *Olpidiopsis* species and this may have influenced somewhat Barrett's interpretation of certain paired nuclei which he observed and illustrated.

Inasmuch as karyogamy in the incipient resting spores of *Olpidiopsis* has not been proven, the true significance of plasmogamy, which has been so often observed and discussed, and the importance of *Olpidiopsis* species in taxonomic considerations remain unknown. Consequently, a general cytological study of *O. Achlyae* has been made with particular reference to the formation of its sexual and asexual resting spores.

[THE BULLETIN FOR JANUARY (68: 1–74) WAS ISSUED DECEMBER 31, 1940]

As reported in a previous paper (McLarty 1941), cultures of *Achyla flagellata*, parasitized by *O. Achlyae*, were maintained on boiled hemp seeds in sterile charcoal water. Consecutive stages in the penetration of the host and the subsequent development of zoosporangia and resting spores were studied from the living material in hanging-drop cultures.

For the study of the highly refractive material which is commonly present in the thalli of *O. Achlyae* at all stages of development, osmic acid, Sudan III, xylene, and acetone were used, while the cell walls were tested for cellulose with zinc chloroiodide and Gram's solution accompanied by sulphuric acid.

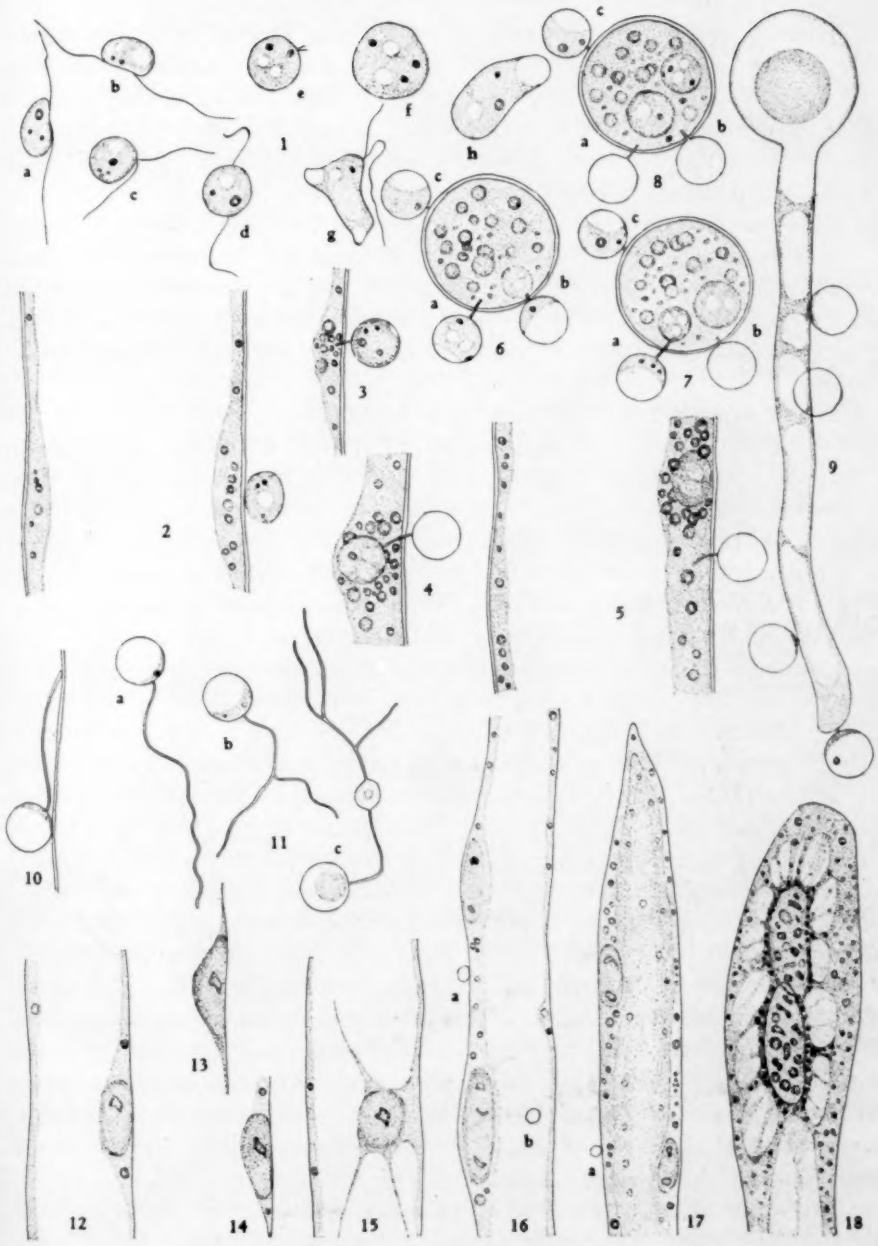
For cytological study of fixed and stained material entire hemp-seed cultures were killed in a variety of standard fixatives at full strength and in various dilutions. Dilute fixatives used for 48 hours gave the best results. Flemming's strong and medium solutions diluted to one quarter and Flemming's weak diluted to one half were most widely used. Nawaschin's fixative gave the best fixation of the host protoplasm. The material was embedded in paraffin, and sections were cut 2–4 μ thick and usually stained with Heidenhain's haematoxylin and Flemming's triple stain. Although the former was useful in the study of the zoosporangium, it was necessary to rely entirely upon the triple stain to differentiate the structures and inclusions of the resting spores.

OBSERVATIONS

The Structure and Germination of the Zoospore. When in motion the zoospore is oval, sometimes reniform in shape, and possesses two equal flagella which are laterally attached near the anterior end (fig. 1*a*, *b*, *c*). In swimming the spore travels with a slight swaying motion as it turns over on its axis in a fashion similar to that described for zoospores of *Thraustotheca clavata* and *Pythiella vernalis* by Weston (1918) and Couch (1935), respec-

Explanation of figures 1–18.

FIG. 1. Zoospores drawn from living material showing refringent globules and vacuoles in finely granular protoplasm; *a–c*, zoospores in motion; *d–g*, zoospores at rest showing the changes in shape, retraction of flagella, and amoeboid movements which may occur. ×1730. FIGS. 2–5. Progressive stages in the penetration of the host by the zoospore and the entrance of the parasite thallus into the host protoplasm. ×1730. FIGS. 6–8. Progressive stages in the penetration of an encysted *Achlya* zoospore by three *O. Achlyae* zoospores. Spore *c* failed to liberate its contents. FIG. 9. A degenerated *Achlya* zoospore which had begun to germinate in water and into which four *O. Achlyae* zoospores had penetrated. FIG. 10. A germinating *O. Achlyae* zoospore whose penetration tube failed to enter the host cell. FIG. 11. *O. Achlyae* zoospores germinating in water in the vicinity of *Achlya* filaments. ×1730. FIGS. 12–15. Progressive amoeboid-like changes in shape brought about in a very young thallus by the streaming of the host protoplasm. ×1270. FIGS. 16–17. Young thalli suspended in the peripheral layer of host protoplasm. ×540. FIG. 18. Two slightly older thalli which have taken up a more or less fixed position in the tip of an *Achlya* filament and have begun drawing the host protoplasm about them. Some localized swelling of the *Achlya* filament is evident. ×540.


a
b
c
d
1
e
f
g
h
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

tively. Upon coming to rest, even momentarily, the zoospore tends to assume a spherical shape (fig. 1*c*, *e*) and at such time the attachment of the flagella at one point can often be observed in living material, although treatment with Gram's solution or osmic acid is often needed to bring out these details sharply. The protoplasm of living zoospores appears faintly brown and slightly granular, with one or several highly refractive, small bodies which give a brilliant fat reaction with Sudan III. In some cases a refringent body is visible at the base of the flagella, as Barrett (1912) has reported.

Zoospores which have been fixed and stained (figs. 44, 45) contain somewhat vacuolate, slightly granular cytoplasm. The most prominent structure in the spore is the large resting nucleus with a prominent central nucleolus. Fairly dense regions in the cytoplasm probably represent remnants of oil bodies.

As the zoospores escape from the mouth of the exit tube they swim directly away without showing any tendency to encyst as do the spores of *Ectrogella, Aphanomycopsis, Pythiella, Achlya*, etc. After an active period varying from a few minutes to one-half hour the spores may come to rest, round up, and in many cases retract their flagella (fig. 1*e*, *f*, *g*). After a period of a few or several minutes, during which the zoospore may assume various shapes (fig. 1*f*, *g*, *h*), it regains its spherical shape, the flagella reappear without change in position, and the spore swims away. Although one or more rest periods may occur during the motile existence of the spore, diplanetism does not seem to be as well defined in *O. Achlyae* as in other members of *Olpidiopsis* (Butler 1907; Barrett 1912). So far encystment of the type found in diplanetic Oomycetes has not been observed in this species. It was originally suggested by Butler (1907) that this occurrence of a period of rest during the swimming period of the zoospores of Chytrideaceous species may represent primitive diplanetism. Supporting this viewpoint, Atkinson (1909) considers diplanetism in certain species of the Chytridiales and Lagenidiales (Karling 1939) to be strong evidence of phylogenetic relationships between the higher Oomycetes and these simpler fungi. The recent discovery of diplanetism in *Ectrogella, Aphanomycopsis*, and *Pythiella* by Scherffel (1925) and Couch (1935) helps to complete the diplanetic series through *Lagenidium* and *Myzocytium* (Zopf 1884) to species of *Pythium* (de Bary 1860) and the higher Oomycetes. At any rate these periodic pauses probably allow the spore to remain viable over greater periods than would otherwise be possible. Zoospores of *O. Achlyae* have been known to remain viable in sterile charcoal for 48 hours.

After swimming for an hour or occasionally for a much longer period, the zoospore settles down on the surface of the host, retracts its flagella (fig. 2), and after exhibiting amoeboid motion for several minutes, rounds up and becomes invested by a definite wall. The protoplasm becomes greyish and

somewhat more granular, and within an hour or less the spore will infect the host or die. A very fine penetration tube is formed at the point of contact with the host (fig. 3), elongates, and usually raises the zoospore slightly from the surface of the host. Penetration appears to take place by local dissolution of the host wall rather than by mechanical action. It is not possible to view the passage of the spore protoplasm through the penetration tube but, under favorable conditions, the movement of the protoplasm out of the spore case and its emergence from the broken end of the exit tube may be observed (figs. 3, 4). After remaining attached for a few minutes, the tiny, slightly vacuolate thallus is carried away in the streaming host protoplasm, where it may be visible for a time before the refringent bodies of the host surround and obscure it from view (fig. 5).

Fischer (1882) suggested that the host may secrete some substance by which the zoospores of the parasite are either repelled or attracted. Zoospores of *O. Achlyae*, germinating in water near *Achlya* filaments, show no tendency to orientate their penetration tubes in the direction of the host. Moreover, abortive penetration tubes have frequently been found growing into empty zoosporangia of the host, while zoospores in contact with the host may produce long penetration tubes which fail to penetrate (fig. 10). This does not appear to be due to rigidity of the wall of the filament, for penetration of encysted zoospores of the host by *O. Achlyae* spores has often been observed (figs. 6–8).

So far it has been impossible to germinate the zoospores on any artificial medium. Spores were often observed, however, germinating in water in the vicinity of *Achlya* filaments (fig. 11), producing long, slender, simple or branched penetration tubes which failed to open. Some such spores (fig. 11*c*) appear similar to germinating spores of *Diplophlyctis, Entophlyctis, Nephrochytrium*, and *Endochytrium* (Karling 1930, 1931, 1938; Hillegas 1940). In the limited number of individuals observed in *O. Achlyae* it was impossible, however, to determine whether or not the nucleus migrated into this swelling on the germ tube as it does in the species referred to above.

Development of the Zoosporangium. About ten hours after penetration, the young thalli become readily visible as rather hyaline bodies of the definite shape, in the peripheral protoplasm of the host. Their protoplasm is very faintly granular and a few highly refractive bodies, which stain brilliant red with Sudan III and blacken with osmic acid, are usually visible (figs. 16, 17). At this early developmental stage no structural, cellulose wall could be demonstrated by plasmolytic methods or by treatment with zinc chloroiodide. The thalli, however, are surrounded by a definite membrane which sharply outlines the parasite and makes it clearly distinguishable from the host protoplasm. The shape which these young thalli assume foreshadows that of the mature sporangium. In this regard the thallus differs markedly from that of

Woronina species (Cornu 1872; Fischer 1882; Cook and Nicholson 1933; and others) in which the plasmodium has been described as being naked and quite indistinguishable from the host protoplasm. The formation of zoosporangia from monospore infections, moreover, shows conclusively that plasmodia are not formed in *O. Achlyae* by the fusion of several thalli within the host cell. This confirms the belief of Fischer (1882) and Barrett (1912).

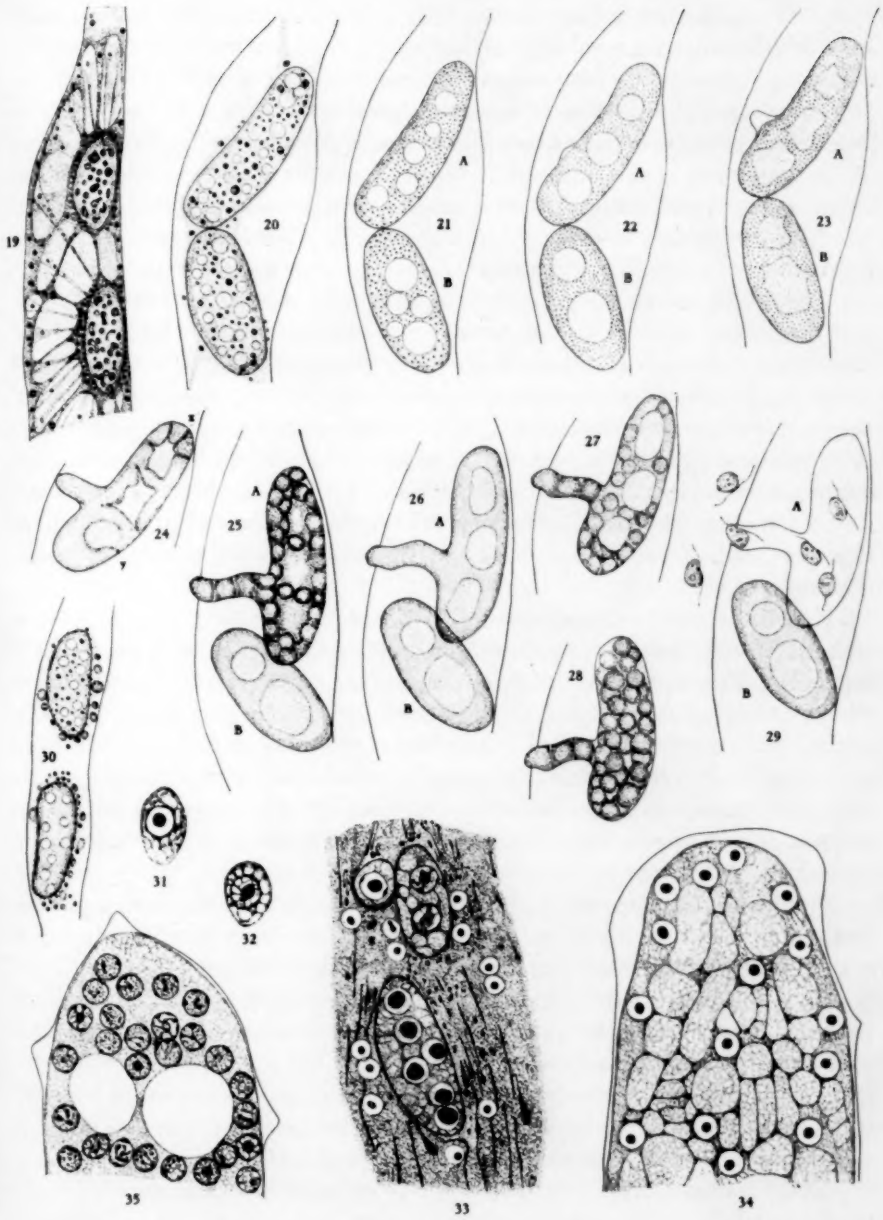
During these early stages of development the thalli behave as passive inclusions in the host and may be carried back and forth in the streaming protoplasm. Depending apparently upon the activity of the host protoplasm, the thalli may develop near the point of infection or they may be carried along and accumulated in localized terminal or intercalary swellings (figs. 18, 19). Fischer (1880), Diehl (1935), and others have maintained that very young thalli of *Olpidiopsis* species may exhibit amoeboid motion. No indication of such activity on the part of young thalli of *O. Achlyae* has been found. Some which remained stationary against the wall of the filament while the host protoplasm streamed rapidly by, exhibited rapid changes in shape and became momentarily elongated in the direction of the flow (figs. 12–15).

When the thalli could be seen clearly, it was obvious that these movements were entirely passive and due to tensions set up by the streaming host protoplasm.

As the thalli increase in size the fatty bodies increase in number and in size and impart a granular, yellowish appearance to the protoplasts which,

Explanation of figures 19–35.

FIGS. 19–29. Progressive stages in the development and maturation of two *O. Achlyae* zoosporangia. ×630. FIG. 19. Two incipient zoosporangia of *O. Achlyae* containing numerous, irregular fatty masses. FIGS. 20–21. Progressive stages in the dispersal of the fatty masses and the concomitant formation of vacuoles in the thalli. FIG. 22. Coalescence of smaller vacuoles to form larger central vacuoles; exit tube of sporangium A is indicated by a slight protuberance. FIG. 23. Sporangia with homogeneously granular protoplasm showing the vacuolar transformations which occur. FIG. 24. Formation of centrifugal cleavage furrows at *x* and *y*. FIG. 25. Sporangium A containing zoospore initials surrounding irregular vacuolar areas. FIG. 26. Sporangia in the homogeneous stage following cleavage during which the lines of demarkation between zoospore segments are invisible; the vacuole-like spaces have regained their even margins. FIG. 27. The reappearance of the zoospore segments by a rounding up of the individual initials. FIG. 28. Zoospores preparing to swarm within the sporangium following the disappearance of the vacuole-like spaces. FIG. 29. Mature zoospores swimming out through the ruptured exit tube of sporangium *A*; sporangium *B*, in the homogeneous stage, is undergoing a period of rest. FIG. 30. Incipient zoosporangia, in the early vacuolate stage, developing fine bristles on their surfaces. ×540. FIGS. 31–32. Very young uninucleate thalli of *O. Achlyae* stained with haematoxylin. ×1660. FIG. 33. Three young thalli of the parasite floating freely in the host protoplasm; lines of flow indicate that the latter was actively streaming when fixation took place. ×1660. FIG. 34. A slightly older sporangium stained with haematoxylin showing resting nuclei suspended in finely granular protoplasm which exhibits irregular spaces which were occupied by fatty masses in the living condition. ×1660. FIG. 35. A portion of a sporangium which was fixed in an early stage of vacuole formation. ×1660.


19
20
A
21
B
A
22
B
A
23
B
24
25
A
B
26
A
B
27
28
A
B
29
30
31
32
33
34
35

at the same time, become surrounded by a membrane which gives a positive cellulose reaction with zinc chloroiodide. In the meantime the host filament usually becomes enlarged around the thalli, and the host protoplasm becomes highly vacuolate with radially arranged cytoplasmic strands (figs. 18, 19) connecting the peripheral host protoplasm with the dense layer of degenerating protoplasm immediately surrounding the parasite. As the thallus continues to grow streaming of the host protoplasm may be observed and granules may be seen flowing in toward it from all sides. Finally all the visible host protoplasm is absorbed by the thallus. Within the parasite the refringent bodies gradually become broken up and appear as oily droplets suspended in a more homogeneous protoplasm. At this stage of development, small vacuoles make their appearance in the thallus (fig. 20). As these vacuoles become more distinct they begin to coalesce, while the dispersion of the fatty material becomes more complete. At this stage the protoplasm of the parasite appears granular and light brown in color, owing to the small, fatty bodies dispersed throughout it. Sudan III when applied to such thalli stains the protoplasm brick-brown. Cook and Nicholson (1933) demonstrated that fatty material in *Woronina polycystis* became converted into proteins at this stage. Millon's reagent, however, failed to indicate a similar condition in *O. Achlyae*.

Up to this point the elaboration and dispersion of fatty material in the sporangia of *O. Achlyae* is essentially similar to what has been described in most chytrid species. In the latter, however, coalescence of the droplets occurs before cleavage takes place. Consequently the protoplasm becomes hyaline and each zoospore receives one large refringent globule. In *O. Achlya*, as well as in other members of the genus and in Saprolegniaceous species, the refringent bodies do not coalesce and, as a result, when zoospore initials are delimited, each one includes several tiny droplets which impart a granular appearance to the protoplasm.

Further developmental stages of these two thalli were observed and illustrated at various time intervals (figs. 21–29). At 10:00 p.m. vacuoles were clearly visible in the thalli (fig. 21). Fusions between vacuoles occurred until, at 10:16 p.m., the thalli appeared as illustrated in figure 22. At the same time the rudimentary exit tube made its first appearance on sporangium *A* as a small, hyaline protuberance and began to grow toward the host wall. Fifty minutes later the vacuoles began to undergo changes in shape (fig. 23) which continued for fourteen minutes before the cleavage furrows began to run out from the vacuoles to the plasma membrane (fig. 24*x*, *y*).

At this point our observations differ from those of Schwarze (1922) for *O. Saprolegniae* in that the spaces previously occupied by the vacuoles did not disappear when the cleavage furrows cut through the plasma membrane. Consequently, for about one minute following the completion of cleavage,

the zoospore initials remained faintly visible flanking the irregular vacuolar spaces (fig. 25*A*). Then, as the protoplasm became homogeneously granular and somewhat oleaginous, the outlines of the zoospore initials disappeared and the vacuole-like spaces regained their even contours (fig. 26*A*, *B*). During the process of cleavage there was no indication of loss of water or fatty material from the spore segments comparable to what Harper, Bold, and others describe. The disappearance of the lines of demarkation, however, is probably due to increase in size through rehydration as Harper (1899, 1914), Schwarze (1922), and Karling (1937b) have described.

While these developments were taking place within the sporangium, the exit tube continued to grow until it came in contact with the host wall (fig. 24). Subsequent growth of the tube caused the sporangium to shift its position in the filament although the latter remained free in the hypha and did not become lodged against the opposite wall. Consequently, the tip of the exit tube could not exert pressure on the host wall. Furthermore, no appressorium-like attachment organ, which might have allowed the tube to pierce the filament mechanically, was observed. The host wall appeared to dissolve at the point of contact with the exit tube and the latter moved through to assume the position shown in figure 25*A*.

Sporangia in the condition illustrated in figure 26*A*, *B* may undergo a prolonged rest period before liberating their zoospores. That the segments do not become confluent during this rest period, as Büsgen (1882) and Butler (1907) believed, may easily be demonstrated by slightly plasmolyzing sporangia in this condition.

After a rest period of 43 minutes the spore segments became visible again (fig. 27). Twenty minutes later the vacuole-like spaces suddenly disappeared and the spores, becoming somewhat larger and assuming their mature shape, began to swarm in the sporangium (fig. 28). After a swarming period which lasted only for three minutes the tip of the exit tube burst and the zoospores emerged fully formed and swam away (fig. 29*A*). The spores may swarm in the sporangium for thirty minutes or more.

The first spores to leave the sporangium seem to be forcefully ejected by pressure within it sufficient to burst the exit tube and to expel an initial mass of spores a considerable distance from the end of the exit tube. It has been noticed that bacteria tend to accumulate around the tips of newly opened exit tubes and subsequently to infest the empty sporangia. This suggests the presence of some attractive, hyaline osmotic fluid in which the spores have been bathed. Karling (1937a) observed similar hyaline material accompanying the zoospores of *Endochytrium operculatum* as they emerged from the sporangium.

As has been pointed out above, the piercing of the plasma membrane by the cleavage furrows in sporangia of *O. Achlyae* does not lead immediately

to the loss of the vacuolar sap and the consequent disappearance of the vacuolar spaces. Bold (1933) likewise illustrates a vacuole-like space surrounded by multinucleate segments of protoplasm which have been cut out by the primary cleavage furrows in *Protosiphon botryoides*. As is shown in figures 41 and 42 from stained material, neither a continuous plasma membrane nor a tonoplast bounding the vacuole-like spaces is demonstrable after cleavage has been completed. In spite of this, the sporangia of *O. Achlyae* have been found to maintain their size and, when the zoospores are liberated, have shown evidence of osmotic pressure within the sporangium.

When fixed and stained, the protoplasm of young, uninucleate thalli appears finely granular and rather vacuolate (figs. 31, 32). In figure 33 a uninucleate and a binucleate thallus are shown, with a larger thallus with six nuclei which is presumably an eight-nucleate stage two of whose nuclei lie in another section. This appears comparable to the thalli shown in figures 16 and 17. Although the margin of the thallus is well defined no structural wall is present at this stage of development. The protoplasm of the parasite appears to be fairly vacuolate even though no vacuoles are discernible in the living thalli at this stage. The irregular shape of these vacuole-like spaces, in conjunction with observations on living material, suggests that they merely represent the spaces left in the protoplasm as a result of the dissolution of the fatty material by the fixing agents. A slightly older thallus, probably comparable to those shown in figure 19, is illustrated in figure 34. The thallus now has a definite cellulose wall and the finely reticulate protoplasm contains many irregular vacuole-like spaces which are presumably caused by the distortion and dissolution of the fatty masses during the process of fixation. This is probably the highly vacuolate stage which Dangeard (1890) describes for *O. Saprolegniae*. A stage which is probably comparable to that shown in figure 21 is illustrated in figure 35. The high degree of dispersion of the fatty bodies at this stage of development undoubtedly prevents the distortion of the protoplasm in the course of fixation. Numerous nuclei and clearly defined vacuoles are present in the finely reticulated protoplasm. A later stage, in which all the vacuoles have fused to form a single, large, central vacuole, is shown in figure 37. Judging by the rather uniform distribution of the nuclei throughout the reticulate protoplasm, sporangia in this condition are ready to undergo cleavage. In large sporangia with relatively small central vacuoles, primary cleavage furrows may first cut out large multinucleate masses (fig. 38) as in *Synchytrium decipiens, Pilobolus Crystallinis, Didymium melanospermum* (Harper 1899, 1914) and *Protosiphon botryoides* (Bold 1933). Later uninucleate protoplasts are delimited by secondary furrows. The failure of Schwarze (1922) to believe that such multinucleate masses may be delimited may be due to the fact that he observed cleavage in small thalli similar to those shown in figures 39 and 40. It is to be noted in figure 38 that the nuclei are in the process of division.

A smaller sporangium with a single large central vacuole is shown in figure 39, in which cleavage furrows are evident, arising at the tonoplast as broad V-shaped clefts between pairs of nuclei. At a slightly later stage (fig. 40), the centrifugal furrows have reached the plasma membrane. The uninucleate segments, bordering the vacuolar space, give the latter a scalloped margin similar to that in figure 25*A*. Sporangia fixed during the rest period which follows cleavage are illustrated in figures 41 and 42. Angular, uninucleate segments surrounding clear areas, which are comparable in position to the vacuoles of younger thalli, are clearly evident although no tonoplasts or plasma membranes are discernible. It is to be noted that in his Figure 39, Plate 24, Barrett (1912) has illustrated a similar condition although he does not mention the absence of the tonoplast and describes it simply as a "vacuolate resting condition."

After this period of rest, the segments shrink and suddenly reappear as dense masses of protoplasm. If the earlier stages had not been observed, this phenomenon might give the impression of simultaneous cleavage. The erroneous reports by Barrett (1912) and others of cleavage of this type may be due to a misunderstanding of this stage. When the vacuole-like spaces disappear, the initials enlarge, become somewhat vacuolate (fig. 43), develop flagella, and begin swarming in the sporangium.

Nuclear Division. Nuclear division in *O. Achlyae* is mitotic and, as in the sporangia of most of the lower fungi which have been cytologically investigated, is simultaneous (figs. 37, 75). In the resting condition the nuclei are spherical or slightly oval and contain a centrally placed, deeply-staining nucleolus (fig. 46). Nuclei containing definite nucleoli do not usually exhibit chromatin reticula similar to those described by Karling (1937b) in *Cladochytrium replicatum*. Delicate strands, which with Flemming's triple stain appear blue in contrast to the brilliant red of the nucleolus, have often been observed radiating out from irregular shaped or vacuolate nucleoli (figs. 47–49). In some nuclei with definite, radiating reticula, dense, blue-staining segments have been observed in the periphery of the nucleoli (fig. 49).

These observations suggest that the nucleolus in this species functions as a storehouse of chromatin like that which has been described by Wager (1913), Curtis (1921), Kusano (1907a, 1907b, 1930) in *Polyphagus Euglenae, Synchytrium endobioticum, S. Pueriae,* and *S. fulgens.* Although definite extrusions, similar to those described by Miss Curtis, have not been seen, the orientation of the chromatin net work upon the nucleolus, the vacuolation and differential staining properties of the latter, together with the early disappearance of the nucleolus during mitosis suggest, at least, a close association of the latter with the formation of the chromosomes. In *Cladochytrium replicatum* (Karling 1937b) and *Endochytrium operculatum* (Hillegas 1940) the nucleolus has been found to persist well through

the metaphases, apparently taking little or no part in the elaboration of the chromosomes.

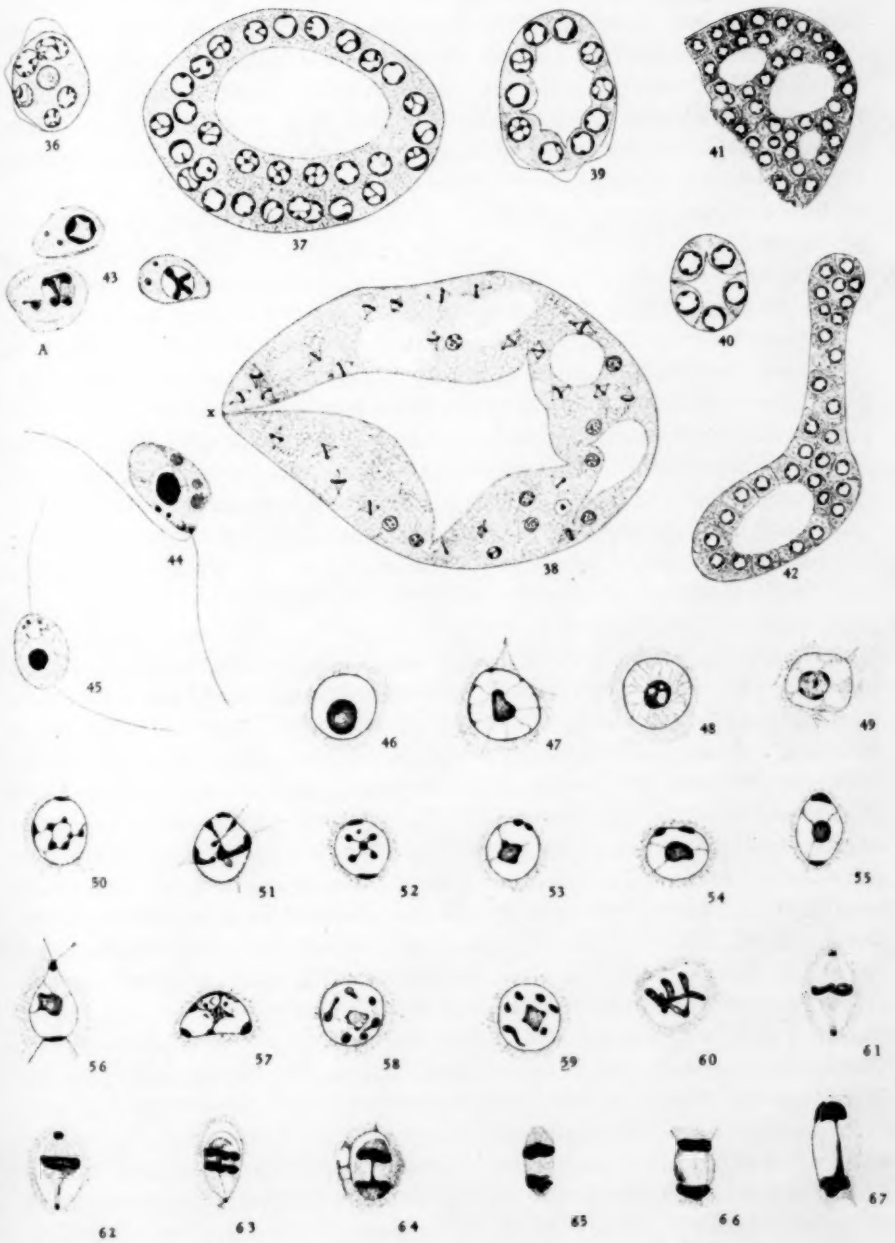
The appearance of radiating chromatin threads has been taken to indicate the beginning of the prophases. In later stages the chromatin is evident as globular (figs. 50, 52) or rod-like masses (fig. 51) distributed throughout the karyoplasm. In late prophase the nucleolar material is usually absent.

Concomitant with the formation of the chromosomes is the production and division of a centrosome-like body similar to that described by Harper (1895, 1905) for *Erysiphe* and *Phyllactinia* (fig. 50, 53–57). The chromatin material, however, has shown no tendency to be orientated upon these bodies nor has any marked development of astral radiations been observed (fig. 56).

The chromosomes, at the close of the prophases, become arranged in a ring about the margin of the equator of the spindle. In some nuclei this arrangement is evident in profile views of the metaphases (fig. 62), but most commonly the chromosomes are crowded together and appear as a dark line (fig. 61). At this stage the nucleus becomes somewhat elongate and the centrosomes at the poles of the spindle are clearly evident.

Explanation of figures 36–67.

FIG. 36. A small sporangium containing five nuclei, two of which are unusually large. × 1655. FIG. 37. A sporangium nearing maturity with a single large vacuole and reticulate protoplasm with numerous, rather evenly spaced nuclei. × 1655. FIG. 38. Primary cleavage furrows delimiting multinucleate protoplasmic masses. × 1170. FIGS. 39–40. Small sporangia stained with Flemming's triple stain showing the origin of the cleavage furrows at the tonoplast and their progressive centrifugal development out to the plasma membrane. × 1655. FIGS. 41–42. Zoosporangia which were fixed during the homogeneous stage and stained with haematoxylin. × 1170. FIG. 43. Incipient zoospores of *O. Achlyae* fixed within the sporangium and stained with Flemming's triple stain to show distinct chromatin masses scattered throughout the nuclei; nucleolar material is present only in zoospore *A*. × 2150. FIGS. 44–45. Mature zoospores of *O. Achlyae* with conspicuous nucleoli within the nuclei and very little chromatic material. × 2150. FIG. 46. Resting nucleus of *O. Achlyae* with a prominent central nucleolus surrounded by hyaline karyoplasm. × 3100. FIG. 47. An early prophase stage with an irregular nucleole from which faint chromatin strands radiate. × 3100. FIG. 48. A prophase stage showing a chromatin network surrounding a vacuolate nucleole. × 3100. FIG. 49. A prophase stage stained with Flemming's triple stain showing chromatin material surrounding a ruby red nucleolus in which blue crescentic chromatin masses are evident. × 3100. FIGS. 50–51. Prophases with spherical and rod-like chromatin masses, respectively, without nucleolar material; a single central body is present on each nuclear membrane. × 3100. FIG. 52. A prophase stage showing six chromatin masses and two polar bodies on the nuclear membrane. × 3100. FIGS. 53–55. Stages in the division of the central body and the migration of the daughter bodies to the poles of the nucleus. × 3100. FIG. 56. A prophase stage showing polar bodies with a few astral rays. × 3100. FIG. 57. A nucleus with central bodies arriving at the poles and with scattered chromatin masses. × 3100. FIGS. 58–59. Polar views of equatorial plate stages with six chromosomes arranged in a ring. × 3100. FIG. 60. Polar view of an equatorial plate stage showing six elongate chromosomes. × 3100. FIGS. 61–62. Profile views of equatorial plate stages showing central bodies at the poles of the spindle. × 3100. FIG. 63. An early anaphase stage; a central body is shown at one pole of the spindle. × 3100. FIGS. 64–66. Progressively later anaphase stages. × 3100. FIG. 67. Late anaphase showing the elongate spindle with what appears to be a remnant of the nuclear membrane to one side. × 3100.


36
37
39
41
43
A
40
x
38
42
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67

In polar views of the metaphases (figs. 58–60) six chromosomes in a ring have often been observed. In all favorable preparations studied so far the chromosome number has been constant and the same as the count which Barrett (1912) made for the chromosomes of *O. vexans*. No indication of reduction division has ever been encountered throughout this investigation. It is probable that reduction division in *Olpidiopsis* species takes place at the time of germination of sexual resting spores.

The earliest anaphase which has so far been observed, is illustrated in figure 63 and the chromosomes, which have separated slightly, form two parallel lines near the equator. Later anaphase stages are shown in figures 64–67. Barrett (1912) found that the nuclear membrane in *O. vexans* disappeared during the late anaphase stages. The achromatic spindle in such case would be intranuclear in origin as in most chytrid species which have been examined. In *O. Achlyae* the nuclear membrane may be rather indistinct in the metaphases but the nuclear area is sharply set apart from the surrounding cytoplasm. Although fragments of the membrane may be present during the anaphase stages (fig. 67) the breakdown of the nuclear membrane in *O. Achlyae* apparently takes place during the late metaphase stages. In the late anaphases the spindle becomes much more elongate (fig. 67), forcing the masses of chromatin farther apart. This elongate spindle seems to be typical of the lower fungi and has been described by Blackman (1904), Barrett (1912), Wager (1913), Dodge and Gaiser (1926), Karling (1937*b*) and others for various species of chytrids, smuts, and other fungi.

The formation of the daughter nuclear membranes in the telophases was not observed nor were the stages of interphase reorganization of the nuclei followed. It was found, however, that the nuclei in cleaving sporangia (figs. 39, 40) and in zoospore segments (figs. 41, 42) were uniform in their internal organization and appearance. It was assumed that these are the daughter nuclei of the final nuclear divisions in the sporangia and that they represent telophasic reconstruction stages. In all such preparations studied, the nuclei, when stained with Flemming's triple stain, exhibit chromatin masses about the inner margin of the nuclear membrane with some scattered strands traversing the nuclear cavity (figs. 39–42). No nucleolar material has been observed in any preparations of sporangia in these stages of development. The nuclear organization in immature zoospores (fig. 43) is similar to that just described although (fig. 43*A*) indefinite bodies which take a faint safranin stain have been observed in the nuclei of zoospores which had been fixed while still in the sporangium. Mature zoospores (figs. 44, 45) usually contain nuclei with large, central nucleoli and little, if any recognizable chromatin material. These appearances suggest that the reorganization stages are the reverse of the prophases and that the chromosome substance becomes largely transformed into a nucleolus as Bally (1911), Kusano, and Miss Curtis have described for species of *Synchytrium*.

Development of the Resting Spore. The early developmental stages of the incipient resting-spore thalli appear to be identical with those of the zoosporangia, and it is not until the former have attained considerable size that they are distinguishable. In figure 68 a swollen filament containing two female thalli, one small male cell, and two incipient zoosporangia is illustrated. The female thalli are filled almost completely with conspicuous, highly refractive bodies and are consequently recognizable. The male cell, however, is distinguishable from the zoosporangia only by virtue of its association with the female thallus.

At this stage, previous to the deposition of the exospore, the highly refractive bodies in the female cells stain brilliant red with Sudan III, thus indicating their fatty nature.

In fixed and stained material the female cells are easily recognized by their staining reactions. Varying numbers of spherical bodies, apparently the stroma of fatty masses, are present in thalli fixed at a fairly early stage. These bodies, which usually stain brilliantly with crystal violet, are absent in zoosporangial thalli. With Flemming's triple stain these bodies have a translucency which sets them apart from the remaining contents of the thalli, while with Heidenhain's haematoxylin they show greater affinity for the stain and consequently render the preparation difficult if not impossible to study.

A stained preparation of a young female thallus, somewhat younger than those shown in figure 68, is illustrated in figure 73. Nuclei in the prophases of division are scattered throughout the finely reticulate protoplasm in which a few brightly staining bodies, interpreted as the stroma of fatty masses, are dispersed. A slightly older thallus is shown in figure 74 in which the protoplasm exhibits a mesh-like configuration which seems to be associated with the appearance of fixed protoplasm from which large globules of fat or oil have been dissolved during fixation. The exospore is developing around this thallus, and numerous granules of the host protoplasm are present. Dark-staining lines of deposition may be seen extending centripetally within the exospore.

Successive stages in the development of the thalli shown in figure 68 are shown in figures 69–72. In figure 69 some tendency for the fatty masses to increase in number and decrease in size is noted. Hyaline and slightly amber-colored amorphous layers have appeared around the female thalli, replacing in part the dense layer of host protoplasm.

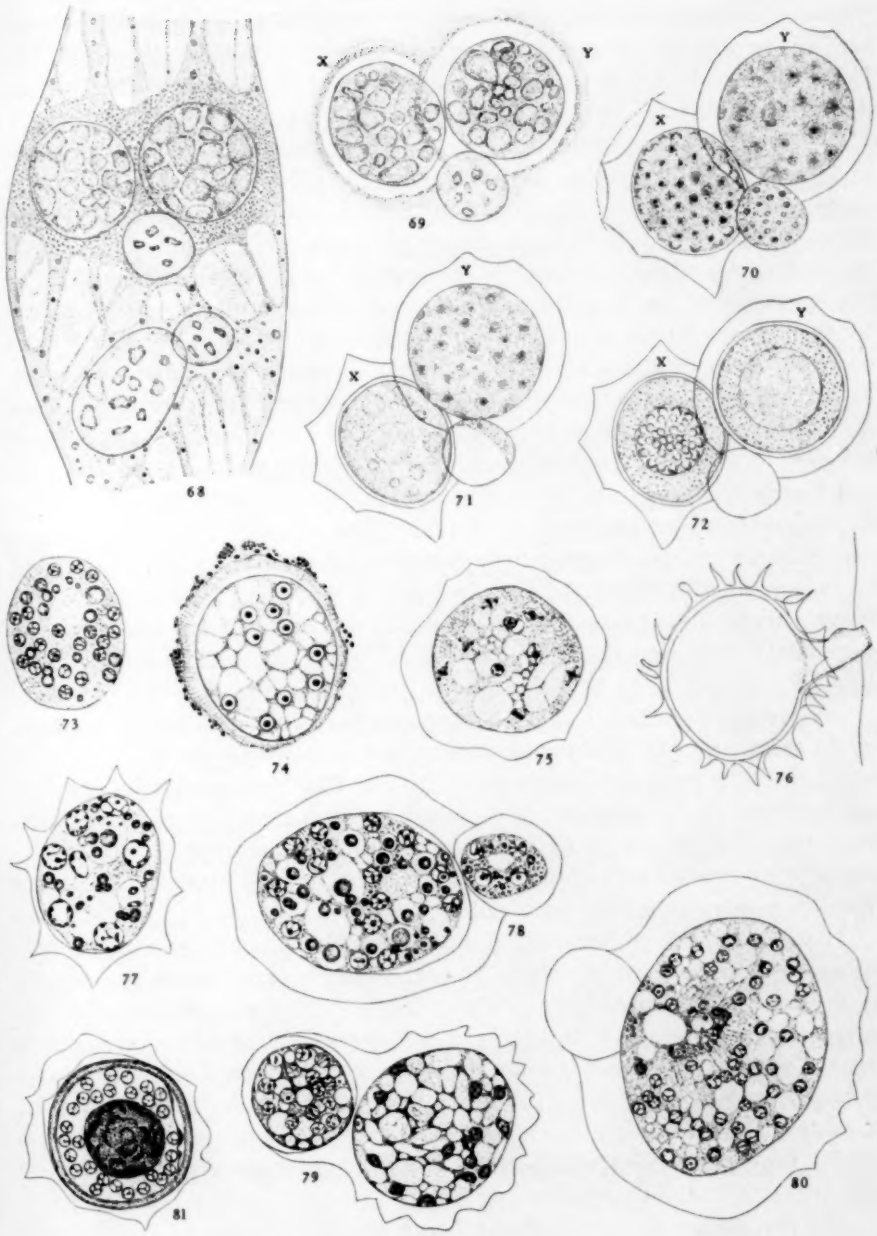
In figure 70 all the host protoplasm surrounding the female thalli has disappeared and the latter are now enveloped by thick, hyaline layers which constitute the exospores. In sporangium *X* several broad-based spines have been evolved within the area of the amorphous layer and the outer limit of the latter is indicated in part by a faint line joining the apices of three of

the spines. In the same figure the first stage in the development of the endospore is visible. This appears to first involve a retraction of the granular contents from the original wall to form a hyaline layer which eventually becomes a definite, double-contoured wall (fig. 71*X*). The contents of the female thalli have become more dense and finely granular by the progressive dispersal of the fatty masses shown in figure 69. The male cell likewise contains numerous small refringent bodies. At this stage the fusion of the thalli is about to occur. The highly vacuolate condition of the male and female thalli described by Barrett (1912) has seldom been observed.

Figure 75 illustrates a haematoxylin-stained preparation of a spore comparable to that shown in figure 70*X*. The nuclei are in the equatorial plate stages of division. Vacuole-like spaces are evident in the protoplasm which doubtless represent the regions previously occupied by fatty material. There is no indication of the exospore in this preparation. Figures 78 and 79 represent incipient sexual resting spores stained with Flemming's triple stain which are comparable to figure 70*Y* of living material. The multinucleate protoplasm of the female thallus shown in figure 78 contains irregular vacuole-like spaces, similar to those described above, some of which seem to contain spherical bodies which have been interpreted as the stroma of fatty masses. These may be the bodies which Barrett (1912) described in the vacuoles of fixed and stained resting spores of *O. luxurians*. The nuclei of

Explanation of figures 68–81.

FIGS. 68–72. Progressive stages in the development of an asexual resting spore (*X*) and a sexual resting spore (*Y*). ×630. FIG. 68. Female thalli containing large, irregular fatty masses; the male thallus is similar in appearance to the zoosporangial thalli near by. FIG. 69. The male thallus remains unchanged while the fatty masses in the female thalli *X* and *Y* have become smaller and more numerous; the thalli are surrounded by a hyaline layer. FIG. 70. The male thallus contains numerous small refringent masses while the female thalli contain dispersed, indistinct oil bodies; the host protoplasm has completely disappeared and the exospores have completely developed; the endospore of resting spore *X* is developing. FIG. 71. Resting spore *X* has a well formed endospore and is filled with homogeneous protoplasm in which spherical fatty masses are visible. The contents of the male thallus are in the process of moving into the female thallus *Y*. FIG. 72. Mature resting spores are with large, central refringent masses. FIG. 73. A young, multinucleate female thallus containing brightly staining bodies which have been associated with the refringent masses of living material. ×1130. FIG. 74. A multinucleate parthenogenetic resting spore of *O. Achlyae;* numerous granules of host protoplasm surround the margin of the exospore, which exhibits centripetal radial lines of deposition. ×1640. FIG. 75. Metaphase plate stages of division of nuclei in an asexual resting spore. ×1140. FIG. 76. An empty, germinated resting spore showing the exit tube. ×620. FIG. 77. A parthenogenetic resting spore showing unusually large nuclei with large chromatin masses. ×1640. FIGS. 78–79. Incipient sexual resting spores stained with Flemming's triple stain showing many nuclei in prophases of division. ×1640. FIG. 80. A sexual resting spore which was fixed just as the contents of the male thallus were moving into the female thallus. ×1140. FIG. 81. A mature asexual resting spore stained with Flemming's triple stain showing the multinucleate, homogeneous, peripheral layer of protoplasm surrounding the central fatty mass; the thallus possesses a smooth endospore and a rough exospore. ×1140.


X
Y
68
69
70
71
72
73
74
75
76
77
78
79
80
81

both the male and the female thalli seem to be in the prophases of division. The content of the former, however, is badly shrunken. In figure 79 a very similar organization of the protoplasts is illustrated. Probably because of the difficulties involved in securing proper fixation of the female thalli after the deposition of the exospore, the nuclei are indistinct and poorly stained.

The passage of the contents of the smaller thallus into the larger is shown in figure 71*Y*. This process may be completed in a few minutes, although the passage of the male protoplast into the female cell may take several hours. There is little change in the appearance of the protoplasts, although in 71*X* the coalescence of the dispersed fatty material has begun in preparation for the formation of the central refringent mass of the mature spore.

Figure 80 represents a sexual resting spore which was apparently fixed while the contents of the male cell were still flowing into the female thallus. From the region of the "fertilization" pore, distinct radiations may be seen which suggest that there were lines of flow in the protoplasm when fixation occurred. As may be observed in figures 78 and 79, the nuclei of the male and female thalli are similar in appearance. Consequently, they are indistinguishable in this preparation. Several pairs of nuclei may be seen but such relative positions appear to be fortuitous. So far, the author has found no indication that the nuclei fuse in pairs as described by Barrett (1912) for *O. vexans*. In some resting spores unusually large nuclei, which at first might have been regarded as fusion nuclei, were observed. The subsequent discovery of such giant nuclei in asexual resting spores (fig. 77) as well as in zoosporangia (fig. 36) indicated, however, that these are merely artifacts.

At maturity, the peripheral region of the spore cavity is filled with homogeneously granular protoplasm with few if any refractive bodies. The center of the spore, however, is occupied by a single, spherical refringent body (fig. 72*Y*) or by an aggregation of many small globules (fig. 72*X*) which, when treated with Sudan III, gives a brilliant red reaction, indicating that it is composed of fatty material.

In figure 81, a fixed and stained median section of a mature, asexual resting spore of *O. Achlyae* is shown. The central area of the spore is occupied by the central body which, with Flemming's triple stain, appears reddish-purple. Surrounding this, in a layer of homogeneous protoplasm, are numerous well defined nuclei containing blue chromatin masses. The exospore and endospore, which have been separated by the microtome knife in the process of sectioning, are clearly distinguishable. The endospore shows a marked affinity for crystal violet, while the exospore fails to stain with haematoxylin and colors very slightly with orange G.

The resting spore, upon germination, becomes transformed directly into a sporangium and liberates zoospores by means of an exit tube (fig. 76) in a manner similar to that described by Shanor (1939) for *O. varians*. Very

few germinating resting spores of *O. Achlyae* have been observed. Consequently, no detailed account of the process of germination can be given.

Host-Parasite Relationship. The penetration of the germ tube into the host protoplasm and the subsequent liberation of the parasitic thallus may cause a localized agitation of the host protoplasm. The refringent bodies of the host protoplasm often swirl in eddies around the young thallus (fig. 4) and obscure it from view. If this may be taken to indicate a distinct reaction between the parasite and the host it is indeed of short duration for, when the thalli next become visible in the filaments (figs. 16, 17), they appear similar to regular inclusions carried about in the streaming protoplasm of the host. A hyaline zone surrounding the parasite, such as Emmons (1931) describes for *Cicinnobolus,* has not been observed. No digestion spaces, described by Smith (1900), Rice (1934), Aronescu (1934), and others in relation to haustoria of *Erysiphe, Puccinia, Diplocarpon,* and other parasitic fungi, have been seen in any stage of development. Three young thalli, as they appear in fixed and stained material, are illustrated in figure 33. Resting nuclei of the host appear quite normal and may be seen in the immediate vicinity of the thalli. No division of the host nuclei in parasitized filaments has been observed, but as Couch (1932), Raper (1936), Wolf (1938), and others have shown, nuclear divisions seldom occur in the distal portions of oomycetous fungi. Consequently, this lack of division stages of the host nuclei is not to be regarded as a reaction to the presence of the parasite. Longitudinal striations in the *Achlya* protoplasm, similar to those illustrated by Hillegas (1940), plate 5, figure 71, indicate that streaming was in progress in the filament when fixation took place.

As the thalli increase in size, however, the "free floating" phase is terminated, and the thalli become relatively localized in the filaments (figs. 18, 19). At this time there is a marked tendency for the protoplasm from all parts of the filament to flow toward and accumulate around the parasites. At the same time hypertrophy of the filament, in the immediate vicinity of the thalli, usually becomes noticeable. As the swelling increases in size, vacuoles appear in the host protoplasm, and radially arranged cytoplasmic strands running from the thalli to the peripheral layer of host plasm may be seen. The granules of the host protoplasm continue to stream into the swollen region of the filament. This movement goes on until all of the visible contents of the entire host filament have been attracted to and absorbed by the developing thallus (fig. 22).

In some instances the swelling of the filament seemed to be initiated in the immediate vicinity of a thallus lying near the host wall (fig. 16). This, however, was by no means the general rule.

Infection with *O. Achlyae* does not invariably bring about an appreciable enlargement of the host filament. The development of sporangia within fila-

ments containing limited amounts of visible protoplasm often occurs without showing the characteristic radial arrangement of the host protoplasm and without causing localized swelling of the filament. Furthermore, in the few infected *Achlya* oogonia which were observed, no swelling or distortion of the oogonial cell was detected. This evidence seems to indicate that the parasite does not cause the host cell to swell by direct stimulation. The parasite seems rather merely to induce the host protoplasm to flow toward it and to accumulate in its vicinity. Consequently, the induction and subsequent growth of the localized swellings appear to be correlated to some degree with the amount of dense protoplasm available in the parasitized filament. The similarities which exist between the formation of these pathological swellings and the development of incipient zoosporangia of *Achlya*, are rather suggestive. In the latter the protoplasm flows rapidly into the tip of the filament, which then swells to accommodate the increased volume. Septation occurs and the young sporangium is cut off from the "sporangiophore," which in turn is left almost devoid of optically differentiated protoplasm. In infection by *O. Achlyae*, the protoplasm of the *Achlya* filament is induced to flow from the more remote parts into the region occupied by the thalli. The filament, consequently, grows in diameter so as to increase its volume locally. No septation of the filament occurs. The walls of the swelling are composed of cellulose and approximate in thickness the walls of normal filaments. This suggests that, if the wall is stretched and becomes thinner as a consequence, this is compensated by a local deposition of new wall material. It can be shown by plasmolytic methods, however, that the wall of the filament is somewhat stretched in the region of such swellings (Diehl 1935).

When infection of an oogonial cell which has been delimited by a cross wall takes place, a clearly defined unit of host protoplasm is involved. The parasite, unable to attract quantities of protoplasm from other parts of the filament, is consequently unable to cause any enlargement of the oogonial cell. Similarly, parasites developing in filaments which contain limited amounts of protoplasm do so without bringing about any appreciable swelling of the host filament.

As mentioned above, the protoplasm of the host may become highly vacuolate in the vicinity of the thalli as the development of the swelling proceeds. Dufrenoy (1936) maintains that such vacuolization may be the result of an effort, on the part of the host cell, to revert to a condition suitable for active metabolism. Although Dufrenoy's interpretation seems to be compatible with the observations of the writer, a thorough physiological analysis must be undertaken before any authoritative statement can be made.

DISCUSSION

Sexual reproduction has now been reported in a large number of chytrid species, but the cytological details in many remain unknown. Resting spores

may arise after planogamic fusions, or as a result of fusions between uninucleate or multinucleate male and female thalli which may take place by means of fertilization pores, or by the production of conjugation tubes. These various types of sexual reproduction, however, do not appear to be confined to any particular family or genus. In the Synchytriaceae planogamic fusions have been described in species of *Synchytrium* and *Micromyces* by Kusano (1930), Köhler (1930), and Couch (1931). Kusano and Köhler believe that sex in *S. fulgens* and *S. endobioticum* is purely relative. In species of the family Olpidiaceae, however, fusions between isogametes have been described by Kusano (1912, 1929) for *Olpidium Viciae* and *O. Trifolii* while fusions of uninucleate male and female thalli have been observed in *Monochytrium Stevensianum* and *Olpidium radicale* by Griggs (1910) and Schwartz and Cook (1928). Planogamic fusions of isogametes have been reported in various Rhizidiaceous species by Scherffel (1925), while Serbinow (1907) describes fusions between uninucleate male and female thalli by means of fertilization pores in *Sporophlyctis rosata*. In the majority of species in this family, however, the passage of the contents from the male into the female thallus takes place by means of a long conjugation canal similar to that which has been observed for *Zygorhizidium* (Lowenthal 1905), *Polyphagus* (Wager 1913), and others. In the Woroninaceae multinucleate male thalli of *Olpidiopsis* (Barrett 1912; McLarty 1941) fuse with multinucleate female thalli by means of a small pore which develops at the point of contact between the two thalli. A similar condition has been described for *Ectrogella* by Scherffel (1925), while in *Pythiella* fusion between thalli is accomplished by the production of a short conjugation tube (Couch 1935).

Almost without exception, sexuality in chytrid species in general does not seem to be fixed and definite. In most species which have been critically examined from the stand-point of sexual reproduction, no evidence of genotypic differentiation of sex has been advanced. In *Olpidium Trifolii*, however, Kusano believes that planogamic fusions occur only between motile cells from different sporangia. Couch (1939) reports heterothallism in *Rhizophlyctis rosea* and *Pringsheimiella dioica*, but no detailed observations have, as yet, been published.

It has been shown by the author (1941) that in *Olpidiopsis Achlyae* genotypic sexual differentiation does not occur. As Barrett (1912) observed for species of this genus which he studied, the male and female thalli appear to be essentially similar to the zoosporangia, differing from the latter mainly in the elaboration and disposition of fatty material throughout the later stages of their development. Plasmogamy occurs in *O. Achlyae* between multinucleate thalli. No nuclear degenerations similar to those described in the sexual processes of many of the higher Oomycetes have been observed

in *O. Achlyae*. Barrett observed what he believed to be stages in the fusion of nuclei in resting spores of *Olpidiopsis vexans*. No indications of karyogamy in *O. Achlyae*, however, have been encountered. In this species, which produces sexual or asexual resting spores, it has been found that "fertilization" is in no way essential to the maturation of the female thalli. At maturity, both sexual and asexual resting spores of *O. Achlyae* are multinucleate and in germination the spores function directly as zoosporangia. In this regard they differ from the uninucleate resting spores of *Polyphagus Euglenae* (Wager 1913), and *Endochytrium operculatum* (Hillegas 1940).

Atkinson (1909) considers that the passage of the entire contents of the male thallus into the larger female cell through a pore without any indication of an antheridial tube indicates that this is a primitive type of sexuality rather than a reduced or degenerated form. The multinucleate condition of the fusion thalli, likewise, has been considered primitive by some observers. Similarly the series from multiple nuclear fusions in *Albugo Bliti* to fusions between single pairs of nuclei in *A. candida* has been considered as exemplifying within a single genus the evolution which has gone on from coenogamic fusions toward the condition which is commonly found in the higher oomycetes. In our present state of knowedge, however, true sexuality may or may not exist in species of *Olpidiopsis*. Until karyogamy is definitely demonstrated in the formation of sexual resting spores of this genus we can do no more than speculate on the relationships between *Olpidiopsis* and the higher fungi.

SUMMARY

The infection of the host by the zoospore of *Olpidiopsis Achlyae* is accomplished by means of a delicate penetration tube. Each uninucleate protoplast thus introduced develops independently into a multinucleate thallus.

Cytokinesis within mature multinucleate zoosporangia is carried out by centripetal cleavage furrows which develop progressively from one or more centrally placed vacuoles. Biflagellate zoospores emerge fully formed from one or more exit tubes.

Nuclear division in *Olpidiopsis Achlyae* is mitotic and simultaneous. The large nucleolus seems to function in the elaboration of the chromosomes. Although central bodies are present at the poles of the intra-nuclear spindle, distinct astral radiations are seldom visible. The chromosome number is six. Meiotic division has not been observed.

The early developmental stages of the resting spore are similar to those of the zoosporangium. Subsequently, however, the resting spore thallus distinguishes itself by the elaboration of large quantities of fatty material. At maturity the resting spore contains a large centrally placed refringent body surrounded by a multinucleate layer of slightly granular protoplasm.

Before the development of the resting spore of *Olpidiopsis Achlyae* fusions between two or more multinucleate thalli may occur. Karyogamy, however, has not been observed.

At maturity, the resting spore functions as a zoosporangium, liberating zoospores directly by means of an exit tube.

The thalli of *Olpidiopsis Achlyae* seem capable of attracting and subsequently absorbing the protoplasm of the entire infected filament. The swelling of the host cell, which usually occurs in the immediate vicinity of the parasite, seems to develop as a result of this local accumulation of excess host protoplasm rather than in response to a direct stimulation of the host by the parasite.

The writer wishes to express his appreciation to Professor John S. Karling, under whose supervision this investigation was conducted.

DEPARTMENT OF BOTANY, COLUMBIA UNIVERSITY
NEW YORK, NEW YORK

Literature Cited

Aronescu, Alice. 1934. *Diplocarpon Rosae:* from spore germination to haustorium formation. Bull. Torrey Club **61:** 291–329.

Atkinson, G. F. 1909. Some problems in the evolution of the lower fungi. Ann. Mycol. **7:** 441–472.

Bally, W. 1911. Cytologische Studien an Chytridineen. Jahrb. Wiss. Bot. **50:** 96–156.

Barrett, J. T. 1912. Development and sexuality of some species of *Olpidiopsis* (Cornu) Fischer. Ann. Bot. **26:** 209–238.

Bary, A. de. 1860. Einige neue *Saprolegnia.* Jahrb. Wiss. Bot. **2:** 169–192.

Blackman, V. H. 1904. On the fertilization, alternation of generations and general cytology of the Uredineae. Ann. Bot. **18:** 323–373.

Bold, H. C. 1933. The life history and cytology of *Protosiphon botryoides.* Bull. Torrey Club **60:** 241–299.

Büsgen, M. 1882. Die Entwicklung der Phycomycetensporangien. Jahrb. Wiss. Bot. **13:** 253–285.

Butler, E. J. 1907. An account of the genus *Pythium* and some Chytridiaceae. Mem. Dept. Agr. India Bot. **1**[5]: 1–160.

Cook, W. R. J., & Nicholson, W. H. 1933. A contribution to our knowledge of *Woronina polycystis.* Ann. Bot. **47:** 851–859.

Cornu, M. 1872. Monographie des Saprolegniées, deuxième partie. Chytridinées parasites des Saprolegniées. Ann. Sci. Nat. V **15:** 112–198.

Couch, J. N. 1931. *Micromyces Zygogonii* Dang., parasitic on *Spirogyra.* Jour. Elisha Mitchell Soc. **46:** 231–239.

————. 1932. The development of the sexual organs in *Leptolegnia caudata.* Am. Jour. Bot. **19:** 584–599.

————. 1935. New or little known chytrids. Mycologia **27:** 160–175.

————. 1939. Heterothallism in the Chytridiales. Jour. Elisha Mitchell Soc. **55:** 409–414.

Curtis, K. M. 1921. The life history and cytology of *Synchytrium endobioticum* (Schilb.) Perc., the cause of wart disease in potato. Phil. Trans. Roy. Soc. London B **210:** 409–478.

Dangeard, P. A. 1890. Recherches histologiques sur les champignons. Le Botaniste **2:** 63–149.

Diehl, H. 1935. Beiträge zur Biologie von *Olpidiopsis Saprolegniae* Barrett. Zentralb. Bakt. Parasitenk. II **92:** 229–249.

Dodge, B. O., & Gaiser, L. O. 1926. The question of nuclear fusions in the blackberry rust *Cacoma nitens*. Jour. Agr. Res. **32:** 1003–1024.

Dufrenoy, J. 1936. Cellular immunity. Am. Jour. Bot. **23:** 70–79.

Emmons, C. W. 1931. *Cicinnobolus Cesatii*, a study in the host-parasite relationships. Bull. Torrey Club **57:** 421–422.

Fischer, A. 1880. Ueber die Stachelkugeln in Saprolegniaschläuchen. Bot. Zeit. **38:** 689–696, 705, 711, 721–726.

———————. 1882. Untersuchungen über die Parasiten der Saprolegnieen. Jahrb. Wiss. Bot. **13:** 286–371.

———————. 1892. Phycomycetes *in* Rabenhorst, L. Deutschlands Kryptogamenflora 1^4.

Griggs, R. F. 1910. *Monochytrium*, a new species of the Chytridiales, its life history and cytology. Ohio Nat. **10:** 44–54.

Harper, R. A. 1895. Beiträge zur Kenntniss der Kernteilung und Sporenbildung im Ascus. Ber. Deuts. Bot. Ges. **13:** 67–78.

———————. 1899. Cell-division in sporangia and asci. Ann. Bot. **13:** 467–525.

———————. 1905. Sexual reproduction and organization of the nucleus in certain mildews. Carn. Inst. Wash. Publ. 37.

———————. 1914. Cleavage in *Didymium melanospermum* (Pers.) Macbr. Am. Jour. Bot. **1:** 127–144.

Hillegas, A. B. 1940. The cytology of *Endochytrium operculatum* (de Wildeman) Karling in relation to its development and organization. Bull. Torrey Club **67:** 1–32.

Karling, J. S. 1930. Studies in the Chytridiales. IV. A further study of *Diplophlyctis intestina* (Schenk) Schroeter. Am. Jour. Bot. **17:** 770–778.

———————. 1931. Studies in the Chytridiales. V. A further study of species of the genus *Entophlyctis*. Am. Jour. Bot. **18:** 443–464.

———————. 1937a. The structure, development, identity, and relationship of *Endochytrium*. Am. Jour. Bot. **24:** 352–364.

———————. 1937b. The cytology of the Chytridiales with special reference to *Cladochytrium replicatum*. Mem. Torrey Club **19:** 1–92.

———————. 1938. A new chytrid genus: *Nephrochytrium*. Am. Jour. Bot. **25:** 211–215.

———————. 1939. A new fungus with anteriorly uniciliate zoospores. Am. Jour. Bot. **26:** 512–519.

Köhler, E. 1930. Beobachtungen an Zoosporenaufschwemmungen von *Synchytrium endobioticum* (Schilb.) Pere. Zentralb. Bakt. Parasitenk. II **82:** 1–10.

Kusano, S. 1907a. On the nucleus of *Synchytrium puerariae* Miyabe. Bot. Mag. (Tokyo) **21:** 118–121.

———————. 1907b. On the cytology of *Synchytrium*. Centralb. Bakt. Parasitenk. II **19:** 538–543.

———————. 1909. Contribution to the cytology of *Synchytrium* and its hosts. Bull. Coll. Agr. Univ. Tokyo **8:** 80–147.

———————. 1912. On the life history and cytology of a new *Olpidium* with special reference to the copulation of motile isogametes. Jour. Tokyo Coll. Agric. **4:** 141–199.

———————. 1929. Observations on *Olpidium Trifolii*. Jour. Coll. Agr. Univ. Tokyo **10:** 83–99.

———————. 1930. Cytology of *Synchytrium fulgens* Schroet. Jour. Coll. Agr. Univ. Tokyo **10:** 347–388.

Lowenthal, W. 1905. Weitere Untersuchungen an Chytridiaceen. Arch. Protistk. **5:** 221–239.

Maurizio, A. 1895. Zur Kenntniss der schweizerischen Wasserpilze nebst Angaben über eine neue Chytridine. Jahresb. Naturf. Ges. Graubündens **38:** 9–38.

McLarty, D. A. 1941. Studies in the Family Woroninaceae–I. Discussion of a new species including a consideration of the genera *Pseudolpidium* and *Olpidiopsis*. Bull. Torrey Club **68:** 49–66.

Minden, M. V. 1911. Chytridineae *in* Kryptogamen Flora der Mark Brandenburg **5:** 209–422.

Raper, J. R. 1936. Heterothallism and sterility in *Achlya* and observations on the cytology of *Achlya bisexualis*. Jour. Elisha Mitchell Soc. **52:** 274–289.

Reinsch, P. 1878. Beobachtungen uber einige neue Saprolegnieae, etc. Jahrb. Wiss. Bot. **11:** 283–311.

Rice, Mabel A. 1934. The relation of *Uromyces Caladii* and other rusts to their hosts. Bull. Torrey Club **61:** 155–162.

Scherffel, A. 1925. Endophytische Phycomyceten-Parasiten der Bacillariaceen und einige neue Monadinen. Arch. Protistk. **52:** 1–141.

Schwartz, E. J., & Cook, W. R. J. 1928. Life history and cytology of a new species of *Olpidium: Olpidium radicale* sp. nov. Trans. Brit. Mycol. Soc. **13:** 205–221.

Schwarze, C. A. 1922. The method of cleavage in the sporangia of certain fungi. Mycologia **14:** 143–172.

Serbinow, J. L. 1907. Beiträge zur Kenntniss der Phycomyceten. Organization und Enwickelungsgeschichte einiger Chytrydeenpilze. Scripta Bot. Horti. Univ. Imp. Petropol. **24:** 149–173.

Shanor, Leland. 1939. Studies in the genus *Olpidiopsis*. I. Resting spore germination in a new species. Jour. Elisha Mitchell Soc. **55:** 167–178.

Smith, G. 1900. The haustoria of the Erysipheae. Bot. Gaz. **29:** 153–184.

Wager, H. 1913. The life history and cytology of *Polyphagus Euglenae*. Ann. Bot. **27:** 173–202.

Weston, W. H. 1918. The development of *Thraustotheca*, a peculiar water mold. Ann. Bot. **32:** 155–173.

Wolf, F. T. 1938. Cytological observations on gametogenesis and fertilization in *Achlya flagellata*. Mycologia **30:** 456–467.

Zopf, W. 1884. Zur Kenntniss der Phycomyceten. Nova Acta Leop.-Carol. Deuts. Akad. Nat. **47:** 143–236.

STUDIES IN THE ERICALES. A DISCUSSION OF THE GENUS BEFARIA IN NORTH AMERICA

W. H. Camp

INTRODUCTION

Befaria,[1] like many woody genera present in North America, has its greatest accumulation of species in South America. In consequence, one hesitates to undertake a critical study of the North American forms without having first solved the complexities of the genus as it occurs in South America. Owing to present world conditions and the concomitant difficulties of travel and specimen exchange, various critical collections cannot be examined, which forces postponement of the nomenclatural phase of the problem as it relates to the South American members of the genus. However, sufficient specimens are at hand to enable one to arrive at some conclusion about the morphological archetypes of the group as well as their distribution. Therefore, in order to understand those of our own species which are taxonomically troublesome, an attempt is here made to fit them into the species-pattern of the ancestral forms entering the area. While this procedure does not make the identification of any particular specimen any easier, it does bring to our study a certain perspective not otherwise obtained.

It is evident that the genus *Befaria* has not, in some characters, advanced nearly so far as others of the Ericaceae; that, while it is not to be thought of as ancestral to its relatives, it yet retains certain of the floral sructures which must have been characteristic of the primitive Ericalean type. In addition to discrete rather than fused corolla segments, the character perhaps most indicative of a retained primitive condition is the often variable number of flower parts in each cycle, this in itself being an indication that the basic genetic pattern is neither rigidly controlled nor highly evolved. A further indication of primitiveness is the presence, in many species, of a glandular pubescence on various plant organs, a feature common to the majority of the more primitive members of various groups of the Ericales.

It would appear that during the Early Tertiary or perhaps even the latter part of the Cretaceous the genus had much the same general distribution as today, but it is probable that the number of species was considerably less than that recognized in the most recent monograph.[2] It is also appar-

[1] The question as to whether the spelling should be *Befaria* or *Bejaria* is not taken up here; it is of no importance in the present discussion, the two being considered only variant spellings of the same name. For those interested in such matters, reference may be made to Small, N. Am. Flora **29**: 35. 1914 (*Befaria*); Fedtschenko and Basilevskaja, Bot. Gaz. **85**: 299. 1928 (*Bejaria*); Sprague, Kew Bull. **1928**: 347. 1928 (*Bejaria*); Mansfeld and Sleumer, Notizbl. **12**: 242. 1935 (*Befaria*); as well as the earlier writers cited by these authors.

[2] Mansfeld, R. & Sleumer, H. Revision der Gattung *Befaria* Mutis. Notizbl. **12**: 235–276. 1935.

ent that both during and subsequent to the various orogenic and subsidence cycles of the Middle and Late Tertiary the distributions of various basic species of the genus were disorganized.

BEFARIA IN THE UNITED STATES

In a genus where specific lines are often difficult to determine, the lack of individual variability is perhaps the most striking feature of *B. racemosa* Vent., a species fairly abundant throughout much of Florida, with casual outliers in Georgia and a single specimen (doubtful) from "Mobile" [Alabama?] (*Cozzens* in 1826). In fact, the specimens are so nearly similar that one might suspect that they all came from the same clone.

The stable condition of *B. racemosa* is probably due to its long isolation from any other of the Befarian stocks. Only recently have students of the flora of the southeastern coastal plain of the United States realized that a portion of Florida has been emergent since at least the Oligocene. It is becoming continually more obvious that this island, for a considerable length of time isolated from both the mainland to the north and the main mass of Antillia (but connected with this latter mass during the Early Tertiary), has played an important rôle in the composition of our present-day southeastern coastal plain flora.

Prior to the Oligocene the exact story of this relatively small bit of land is obscure but, being connected with the western portion of Antillia probably during at least the latter part of the Cretaceous, this area (now part of Florida) was one into which the early tropical and subtropical lowland floras could migrate. From the Oligocene until the late Pliocene, or even the Pleistocene, it was an island separated from the mainland of North America by several hundreds of miles of open ocean. Thus, lying on the outer fringe of the primary Befarian dispersal and, from at least the Oligocene to the Pleistocene having been isolated on a comparatively small island, it is only natural that *Befaria racemosa* should be practically homozygous and therefore morphologically stable.

BEFARIA IN THE CARIBBEAN

Throughout at least part of the Tertiary, Antillia was joined with both South America and Central America. But this great land mass, with its east-west-trending ranges of mountains, was on the outer fringes of the distribution of *Befaria* and, as a result, held only a small segment of the genus. Today, we know only one species from the interior of the Antillean area, *Befaria cubensis* Griseb., from western Cuba. However, it is possible that subsequent exploration on the higher peaks of Oriente in Cuba, or even in Hispañola, may uncover material as yet unknown to us. *B. cubensis*, although today a distinct and reasonably stable species, is reminiscent of the

Venezuelan and Colombian *B. ledifolia* H. & B., and seems to have had genetic connection with it in the past. It is also possible that the tendency for revolute leaves in many of the South American forms may have been derived from this segment of the older Befarian complex.

THE PROBLEM IN BEFARIA

If we now turn our attention to the bulk of the known Mexican species and those southward into South America, no such stability of morphological characters is to be noted as was found in *B. racemosa*. Nevertheless, if we are ultimately to understand the genus, if we are to rationalize our present nomenclatural system with the fundamental biology of the group, we must first arrive at a rather definite concept of its primitive biotypes as well as their distribution. It is to be admitted that, for the present, a complete picture of the genus is impossible, simply because certain critical areas are in need of considerable exploration. Nor am I as yet ready to commit myself in a definitive manner concerning the exact pattern of speciation within the South American portion of the genus.

However, it is apparent that there have been several evolutionary trends in *Befaria*. One was a reduction of the more primitive, loosely flowered, racemose-paniculate type of inflorescence to a more compact and congested inflorescence. Others are the loss of the primitive type of Ericalean pubescence which resulted in a glabrous plant, and the introduction of revolute margined leaves. These evolutionary trends appear to be completely independent of each other. From this standpoint, it would seem that, after their earlier partial stabilization, the primary species-populations have, in certain areas, been mixed. This has resulted in a series of hybrid swarms of comparatively recent origin; complex populations of plants, the individuals of which apparently defy arbitrary placement in traditional taxonomic categories.

THE ARCHETYPES OF THE MEXICAN UPLANDS

Of the Mexican species of *Befaria* here recognized, only two seem to be representatives of an early dispersal.[3] The remaining appear to be of more recent origin.

[3] It is possible that an additional species may yet be collected in Mexico, and attention should be called to the following: In December, 1936, I traveled from Veracruz to Oaxaca by way of Orizaba. Before the mountains were reached and while yet in the *tierra caliente* of the State of Vera Cruz, the train passed through a few inland swamps wherein some shrub with conspicuous flowers was occasionally seen. In one of these areas the train was traveling rather slowly and I was able to note the habit of the plants growing nearby. This shrub apparently was a species of *Befaria*. Unfortunately, I was unable to visit this area on my return in 1937; neither have I seen specimens from this region nor, so far as I can learn, have any been collected. These plants most certainly were not *B. mexicana*, having, if my memory can be trusted, an inflorescence somewhat intermediate between the paniculate type of *B. glauca* and the racemose appearance of *B. racemosa*.

Of these basic species,[4] the first to be considered is *Befaria mexicana* Benth. This species ranges in Mexico from Durango [?] (*Seemann 2130* is so listed by Mansfeld and Sleumer) and Zacatecas (*Hartweg 92*, type collection) southward and eastward through the Sierra Madre of Jalisco (*Mexia 1840*) and Guerrero (*Mexia 8956; Hinton 14106, 14200, 9935*, this last in Herb. Field Mus.) to Oaxaca (*Conzatti 2530*, N. Y. and Herb. Field Mus., *4355;* and *Schultes & Reko 939.*) Material from the northwestern part of the range apparently has larger corollas (± 3 cm. long) and is more consistently pubescent than that in Oaxaca, being thus closer to the typical form.

The other primary type with which we are concerned in Mexico is the material which has been formerly referred to *Befaria laevis* Benth. In their recent study, Mansfeld and Sleumer (l.c.) have noted that the Mexican *B. laevis* and the South American *B. glauca* H. & B. var. *typica* Mansf. & Sleum. are difficult to distinguish. In addition, they admit that the primary character (the leaf apex) which they use to separate these species is not reliable. I would go even farther and say that I am unable to differentiate the genetically uncontaminated Venezuelan and Mexican material of these two species. Consequently, I am led to the conclusion that the Mexican material, by previous authors placed in *B. laevis* Benth. (1840), should be transferred to the earlier *B. glauca* H. & B. (1809).

It might, naturally, be argued that a species should not have so disjunct a distribution, but it is well to recall that (even among the Ericaceae) *Befaria* is an old genus and that much of the area of its early distribution has been subject to extensive diastrophic activity and consequent floristic disturbances. Therefore, it is altogether likely that *B. glauca* has been lost from parts of the area where it once grew.

If we examine the known distribution of *B. glauca* in Mexico (*B. laevis* of previous writers) we find that it extends from Chiapas (*Purpus 10285; Matuda 2589*) into Oaxaca (*Hartweg 478*, type coll. of *B. laevis; Ghiesbreght 31*, type coll. of *B. Ghiesbreghtiana;* and *Schultes 677*). Mansfeld and Sleumer (l.c.) list additional specimens from Guerrero and Hidalgo, but *Schultze 468*, the one cited by them as from Guerrero, with white flowers

Occurring as they do in the lowlands rather than in the mountains, the identity of these plants remains an interesting problem, the solution of which may shed considerable light on the early northward dispersal of this genus, for these same lowlands are phytogeographically connected with the western parts of ancient Antillia. In this connection, it is well to remember that, although the genus consists mainly of mountain forms, *B. cubensis* and *B. racemosa* are found only near sea-level.

[4] Unless otherwise noted, the specimens here cited are in the Britton Herbarium of the New York Botanical Garden. I also wish to thank the Curators of various American herbaria who made a fruitless search for certain specimens, and particularly Mr. P. C. Standley of the Field Museum for his kindness in submitting a series of unusually interesting collections.

and petals 3 cm. long certainly cannot be *B. glauca* (or *B. laevis*) in its pure phase. Of the two "Hidalgo" specimens cited by them I have before me *Ghiesbreght 31*, which was not from this state but from Oaxaca;[5] the other (*Seler 866*) I have not seen. I also have before me (without locality) *Sessé, Mocino, Castillo* et *Maldonado 1813* (Herb. Field Mus.). It is obviously conspecific with the others placed here in *B. glauca*.

Before we continue with our analysis of the Mexican species of *Befaria*, it will be advisable first to examine material of these two basic species from areas where it would appear that they have not been subject to interspecific contaminations. Briefly, in Chiapas we find that *B. glauca* has glabrous leaves, glabrous branches, a coarse paniculate inflorescence which, also, except for a minute and early deciduous puberulence, is glabrous. The petals are 1–1.5 cm. long and of a deep pink or rose color. Paler forms and those with larger corollas sometimes found in other regions apparently have a mixed heredity.

Conversely, in Zacatecas, Jalisco, and parts of Guerrero, *B. mexicana* has the leaves glandular pubescent on both surfaces, particularly along the midvein below. The branches are densely glandular pubescent as is the inflorescence which, in contrast to *B. glauca*, is compact and subcorymbose. The petals are 2.5–4 cm. long and white, although pink forms are occasionally recorded. These, in my opinion, may be due to a slight contamination of *B. glauca* heredity. *Mexia 1840* from Jalisco is in fruit.

These very distinct forms seem to represent the two original species present on the Mexican uplands.

THE BEFARIA DISCOLOR COMPLEX

Turning our attention to a region where *Befaria glauca* and *B. mexicana* come together, we are confronted with the following situation: a complex population generally placed in *B. discolor* Benth., but possessing combinatory and segregate forms apparently derived by hybridization from both species. Certain examples, all from the State of Oaxaca, are here briefly analyzed.

Conzatti 2529 (Herb. Field Mus.) with its dense pubescence and petals ± 2.5 cm. long would, in spite of the short corolla, be nearly typical *B. mexicana*, except that the dried plant retains evidence of considerable color in the flower. This number is of particular interest in connection with the previously cited *Conzatti 2530* (collected in the same locality, on the same date), with an apparently white corolla and petals 3–4 cm. long, which was placed in *B. mexicana*.

[5] Mansfeld and Sleumer (l. c.) list *Ghiesbreght 31* from Hidalgo. However, the specimen on deposit at the New York Botanical Garden clearly states that this number was collected in Oaxaca, as does the original description of *B. Ghiesbreghtiana* Fedtschenko & Basilevskaja, Notul. Syst. Herb. Hort. Bot. U.S.S.R. **6**: 38. 1926.

Liebmann 8592 (Herb. Field Mus.) has the branches and inflorescence as pubescent as any specimen of *B. mexicana* that I have seen, yet its corolla was apparently deeply colored; also, the petals are only 2–2.5 cm. long.

The pubescence of *Mexia 9276* is, again, nearly typical for *B. mexicana*, yet the inflorescence form is that of *B. glauca;* the petals are white, but only ± 2 cm. long.

Galeotti 1812 (type coll. of *B. floribunda* Mart. & Gal.) has the branches pubescent, but the pubescence becomes sparse in the inflorescence, with the upper part of the pedicels and calyces completely devoid of the glandular setae so characteristic of *B. mexicana*. The inflorescence resembles that of *B. glauca*, but its individual branches are compacted, much as in typical material of *B. mexicana*. It would appear that the petals were not more than 2 cm. long. The original description states that the flowers are "purpureo-albis"; the field notes list the color as "rosees."

Gómez & Gómez 6000 has almost completely glabrous leaves,[6] the branches are pubescent, but the setae on the pedicels are even more reduced than in the previously cited *Galeotti 1812*, a few flowers having a completely glabrous calyx and only a few scattered hairs on the pedicel. The corolla is ± 2 cm. long and a deep rose-pink color.

[6] *Manuel Gómez & Victoria Conzatti de Gómez* (Herb. Conzatti) *6000* from the District of Teotitlan, Oaxaca, was kindly loaned to me by Richard Evans Schultes, who received it from Professor Conzatti. This collection (the portion studied now deposited in the Economic Herbarium of the Harvard Bot. Mus.) is of particular interest because it is the basis of a new monotypic genus—*Heptacarpus*—(Conzatti, Prof. C. Organo Transitorio en una Talamiflora Serrana. Anal. Hospital Gen. Oaxaca **2**: 4–7. *1 plate.* 1940.). This genus, according to the present International Rules of Botanical Nomenclature, is invalidly published (being without Latin diagnosis); it is also incorrectly placed as to family.

The following personal communication from Mr. Schultes is pertinent to the present discussion and is included:

"In 1839, Hartweg collected the type of *Befaria discolor* at Talea de Castro (Long. 96° 17′, Lat. 17° 21′), District of Villa Alta in northeastern Oaxaca, only a few miles southeast of the locality of collection of *Gómez & Gómez 6000*.

"In the same year, Galeotti made a collection which became the type of *Bejaria floribunda* Mart. & Gal. (= *Befaria discolor* Benth.). This collection (*Galeotti 1812*) was made on the mountains near San Ildefonso Villa Alta (Long. 96° 10′, Lat. 17° 21′) and San Juan Tanetze (Long 96° 19′, Lat. 17° 23′). Villa Alta is slightly east of Talea; Tanetze is but several miles east of Cacalotepec and slightly west of Talea. The specimen in the Gray Herbarium is labelled as coming from the "Chinantla," a name which Galeotti used to designate the mountainous areas of the Districts of Villa Alta and Choapam.

"A third collection was made by Jurgensen in the Sierra San Pedro Nolasco near Talea de Castro. This collection (*Jurgensen 562*) became the type of a new genus—*Jurgensenia*—and species—*J. mexicana* Turczaminov (= *Befaria discolor*).

"These collections, all made within a radius of fifteen miles, have been the basis of material described and placed in two different families—Ericaceae and Theaceae; three genera—*Befaria* (*Bejaria*), *Jurgensenia*, and *Heptacarpus;* and four species—*Befaria discolor, Bejaria floribunda, Jurgensenia mexicana*, and *Heptacarpus salmonicolor*, thus adding to the confusion surrounding *Befaria discolor* Benth."

Hartweg 479 (type coll. of *B. discolor* Benth.) has essentially glabrous leaves, or with a few gland-hairs on the midvein below; the stem has a sparse pubescence, and the pedicels even less. The inflorscence is but little branched, as is common in *B. mexicana*, but it is loose-flowered with spreading pedicels, and thus is much more like that of a single branch of the *B. glauca* type. The calyces are glabrous and the petals 1.5–2 cm. long.

Apparently, then, *B. glauca*, coming from the east, perhaps by way of uplands now submerged, met the Mexican representatives of the *B. mexicana* population and, in the Oaxacan region, produced a series of rather complex hybrid forms. The presence of nearly pure stocks of both species in Oaxaca, as well as these intermediates (today apparently concentrated in the region of the "Chinantla"), indicate that such a sequence of events has taken place.

Our problem, then, is to determine just how we shall treat such a situation. In Mexico, owing to the present isolation of the more mesophytic floras on mountain ranges, or even individual peaks, and these often separated by wide expanses of semi-desert, a considerable amount of endemism is to be expected. With further exploration and a larger series of critically collected specimens, it would not be surprising to learn that certain of these segregates, carrying combinatory characters of both the basic species, have become genetically stabilized and exhibit a reasonably constant set of morphological characters within restricted areas.

For the present, and until we know more concerning them, I see no need to make the problem taxonomically more complex by resorting to a formal description of all of these segregate and morphologically different individuals, thus multiplying our nomenclatural troubles. Instead, it is my suggestion that we consider this problem from the standpoint of expediency; that we do not consider *Befaria discolor* Benth. a true biological species having had a single origin; rather, that it be only a nomenclatural convenience —a name applied to those individuals which, *in sensu stricto*, cannot be placed either in *B. mexicana* or *B. glauca* but would appear to be recurrent segregates of hybrids resulting from crosses between these two species.

A study of the Central American and South American material of *Befaria* indicates that much the same situation exists there as in Mexico involving *B. glauca* and additional species present in the area. Consequently, it is my opinion that Mansfeld and Sleumer (l.c.) have not solved the obviously difficult systematic problem encircling *Befaria* by a traditional taxonomic treatment. This is nowhere better shown than in their disposal of the South American forms allied to *B. glauca*, in which they described four new varieties under this species in addition to the typical form; varieties with monotonously similar distributions and which, when critically examined, clearly indicate hybrid connections with other (and I think valid) species

recognized by them from within the same area. I believe that, if they could be crossed and the resultant hybrids were fertile (which from the material available seems to be the case), one could start with but four of our modern species, *B. ledifolia, B. glauca, B. Mathewsii,* and *B. mexicana* and, in only a comparatively few generations of plants, produce segregate forms indistinguishable from the majority of the species and varieties now recognized in South America. This does not mean that the bulk of these forms are necessarily of recent hybrid origin—although many of them are—but only that, in taxonomic work, one should attempt to distinguish between those which appear to be genetically stable and those which have been given taxonomic rank and placed in the same category merely because of simulative characters—similar forms which are the result of a series of chance segregations from within a hybrid complex. Such so-called "species" are met with all too frequently in the Ericales in such genera as *Befaria, Rhododendron, Gaultheria, Pernettya,* and *Vaccinium.*

However, one cannot leave this problem of the hybrid origin of the Mexican complex, here covered by the name *Befaria discolor,* without certain additional remarks. It is interesting to note that, typically, *B. mexicana* is a "winter bloomer," being in flower from November until late in March, a span of more than four months. Conversely, *B. glauca* is a "summer bloomer," the specimens before me indicating that flowers are present from May into August. It is to be further noted that those specimens placed by me in *B. discolor* span the season of both the other species, flowering plants being recorded at various times from November to June.

It is, of course, well understood that in the tropical uplands the seasonal distribution of rainfall plays an important rôle in the flowering of a majority of species. My own experiences indicate that this is certainly true in the Oaxacan region. However, since there is no great change in the seasonal temperatures, it is not unusual for individual plants to be thrown completely out of their normal flowering period by other factors. Among these may be mentioned unseasonal distributions of rainfall, flower-bud production on the new growth following insect injury and, particularly, the flowering of sprouts after fire. It is to be pointed out in this connection that nowhere in Mexico is the *milpa* type of agriculture more intensively developed than in the mountainous regions of Oaxaca. The burning of these small areas often starts extensive brush fires and, in 1937, to the east of Villa Alta (see footnote 6) on the border of the "Chinantla," while standing in one of these recently burned-over areas, I could see the smoke of at least three such fires on adjoining mountains. Furthermore, this is not a new type of agriculture in the region, having been practiced there for untold centuries.

Therefore, it is probable, although *B. mexicana* and *B. glauca* normally have different flowering periods, that abnormal conditions of one sort or

another have induced individual plants of either species to close the short gap in flowering seasons, thus permitting a mixing of the two species' heredities. It is also evident that such a condition is not limited to the past, but is going on actively at the present time so that, today, we must recognize the presence of current hybrids between these two species as well as a series of segregates from those of the past. It is because of this that I look upon the type of *Befaria discolor* Benth. not as representative of a true biological species but, rather, as only one of a group of divergent forms of complex origin, having in common only a basic bispecific ancestry.

A NEW SPECIES FROM MEXICO

In a previous paragraph I have intimated that it is possible, in certain areas contiguous to the Oaxacan region, that future exploration may uncover populations of individuals, probably of segregate origin, but sufficiently stabilized so that they may be dealt with in a more particular manner. Such entities, while not a part of the ancestral population, might well be thought of as true species—ones which have had their origin in relatively modern times.

I have before me a specimen representative of a group which seems to belong in such a category. It was collected by Mr. Geo. B. Hinton in Guerrero, away from the immediate influence of *B. glauca* and in a region where *B. mexicana* is the dominant form. The collection is from a tree 6 meters tall and, from the material at hand, it would appear that the vegetative parts almost exactly duplicate those of *B. glauca*, although the flowers simulate those of *B. mexicana*, the petals being white and up to 4.5 cm. long. It is also of interest to note that, under *B. laevis* (*B. glauca* of this paper), Mansfeld and Sleumer (l.c.) mention a similar specimen, also from Guerrero (*L. Schultze* Jena *n. 468*), with large flowers, the petals being white and 3 cm. long. I therefore propose the following new species:

Befaria Hintonii Camp, sp. nov. Arbuscula 6 m. alta, ramulis glabris; folia oblongo-lanceolata, utrinque glabra, apice acuta, supra subnitida, subtus glaucescentia, 5–8 cm. longa, 1.5–2.5 cm. lata; inflorescentiae breviter racemosae vel subcorymbosae, 6–12-florae; rhachis pedicellique glabri, pedicellis crassis, 3–4 cm. longis, bracteis parvis deciduis; calyx campanulatus glaberrimus, sub anthesi circ. 8 mm. latu, lobis ovatis obtusis, margine ciliatis; petala alba, obovata-spathulata, basi attenuata, 4–4.5 cm. longa, circ. 1 cm. lata, apice extremo parce puberula; stamina petalis aequilonga; stylus tenuis circ. 5 cm. longus.

MEXICO—GUERRERO: Dist. of Galena, Piedra Ancha, 2800 m., *Geo. B. Hinton 14237*, May 3, 1939. "Oak and pine forest; tree 6 m. high; flower snow white." TYPE in Herbarium of the New York Botanical Garden.

NOMENCLATURAL CHANGES

Befaria glauca Humb. et Bonpl. in Pl. Aequin **2**: 118, t. 117. 1809. Syn. *Befaria laevis* Benth., Pl. Hartweg., p. 65. 1840.

This change enlarges the distribution of the fundamental and once widespread *B. glauca* from South America into Mexico, the genetically uncontaminated Mexican material being scarcely distinguishable from the typical form in Venezuela.

× **Befaria discolor** Benth., stat. emend. Camp. [*Befaria mexicana* Benth. × *B. glauca* H. & B.] *Befaria discolor* Benth. Pl. Hartweg. p. 65. 1840. Syn.: *Heptacarpus salmonicolor* Conzatti, Anal. Hospit. Gen. Oaxaca **2**: 5. 1940. For additional synonyms see Mansfeld & Sleumer (l.c.)

The transfer of *B. discolor* from the status of a true species to that of a name covering a complex series of hybrids and their segregates has been undertaken solely in an attempt to rationalize our present system of nomenclature with what appears to be the biological situation within the material here under discussion. Any other procedure under the existing system would necessitate the giving of some sort of taxonomic standing to the group of names which necessarily would have to be attached to the known morphologically different and describable entities in the complex *Befaria*-population which appears to center in the mountains of northeastern Oaxaca. To do so at the present time would add nothing to our knowledge of the genus and certainly would not clarify its taxonomy.

BEFARIA IN CENTRAL AMERICA

It is of more than passing interest to note that no previous study of the genus has listed *Befaria* from Central America. Fortunately, my attention has been called to four collections from this region recently deposited in the Herbarium of the Field Museum; one from Honduras and three from Guatemala. A genus with such showy flowers is not easily missed by collectors. That it was neither found in Honduras prior to 1936, nor in Gautemala earlier than 1939 indicates either that *Befaria* is exceedingly rare in Central America, or (as I suspect) that the area is in need of considerably more botanical exploration.

These four specimens show some relationship with the Mexican populations, but, owing to certain differences, I am unable to place them in any of these species with confidence.

The single specimen from Honduras (*Yuncker, Dawson & Youse 5823*, July 13, 1936, from a steep ravine near El Achote, hills above the plains of Siguatepeque, Dept. of Comayagua), because of its viscid pubescence, seems to be related to *Befaria mexicana*. However, the leaves are somewhat larger (being up to 10 cm. long) and less glaucous than is typical; the inflorescence is considerably more loose; the pedicels are attenuate, and the calyces only one-half to one-third the diameter they should be for this species. The petals are white and at most 2.5 cm. long, being, thus, just within the lower limit for *B. mexicana*. Although tentatively placed in association with this species, it is not to be thought of as typical.

The three specimens from Guatemala were collected in the Sierra de las Minas, Dept. of Zacapa, in October, 1939. Of these, two (*Steyermark 29775* & *29783*) were from an elevation of 1700–2000 meters. *Steyermark 29783* is in flower and with immature fruit. It was a "tree 30 ft. tall." The plant is glabrous and, in their shape, the leaves closely resemble those of *B. glauca* in its typical phase. The inflorescences are few flowered, the pedicels short and heavy, the calyces fairly large and the flowers white, sweet scented, with petals 2–2.5 cm. long; thus, in these characters it somewhat resembles *B. mexicana*. *Steyermark 29775*, in fruit, has much the same general appearance, but it is recorded as being a "shrub."

The third specimen, *Steyermark 29725*, from a lower elevation (1000–1500 m.), is a "tree 25 ft. tall." The plant is glabrous; the inflorescence is somewhat more diffuse than in the two previously cited specimens; the pedicels and calyces seem to be intermediate in form between those of *B. mexicana* and *B. glauca;* also, the flowers are small, being only 1.5–2 cm. long. The collector has made no note of the flower color, but in the dried specimen the petals are considerably darker than those of his *#29783*. It is probable that, when fresh, they were pink or even red; *#29725*, therefore, seems more closely to resemble *B. glauca* than the other Guatemalan specimens here cited.

In spite of their differences, one character in particular seems to be present in both the Honduran and Guatemalan specimens which, collectively, separates them from those species of the genus to which they might be referred. In general, the lower surface of the leaves of the various species of *Befaria* is definitely glaucous. This is not true of the Central American material available for, although the lower surface of the leaves is paler than the upper, it certainly is not glaucous, at least not in a degree characteristic of those species with which I have acquaintance.

On the basis of *Yuncker, Dawson & Youse 5823* from Honduras, I am predicting that *Befaria mexicana*, or its simulative counterpart, will yet be found in Central America. Also, I am certain that *B. glauca* in its pure form will be collected in Guatemala. This conclusion is reached after an examination of *Steyermark 29725* in conjunction with the distribution of *B. glauca* as here redefined, as well as its known presence in adjoining Chiapas. The remaining material from Guatemala apparently represents a new species.

Befaria guatemalensis Camp, sp. nov. Arbuscula 9 m. alta, ramulis glabris; folia oblongo-lanceolata, utrinque glabra, apice acuta, supra virida, subtus pallida non glaucescentia 5–9 cm. longa, 1.5–2.5 cm. lata; inflorescentiae breviter racemosae vel subcorymbosae 6–10 florae; rhachis pedicellique glabri, pedicellis sub-crassis, 1–3 cm. longis, bracteis parvis deciduis; calyx campanulatus glaberrima, sub anthesi circ. 4 mm. latu, lobis deltoidis, margine ciliatis; petala alba, obovata-spathulata, basi attenuata, 2–2.5 cm.

longa, circ. 0.5 cm. lata, apice extremo parce puberula; stamina petalis aequilonga; stylus tenuis, circ. 3 cm. longus.

GUATEMALA—ZACAPA: pine forest, Sierra de las Minas, near summit of ridge, below Finca Alejandria, alt. 1700–2000 m. *Julian A. Steyermark 29783*, Oct. 12, 1939. "Tree 30 ft. tall. Petals white, sweet-scented." TYPE in the Herbarium of the Field Museum. IBID: *Steyermark 29775*, said to be a shrub and with slightly larger leaves, may be only a sprout form of this species.

Befaria guatemalensis may be easily differentiated from the previously described *B. Hintonii* by its smaller flowers and pale green rather than glaucous lower leaf surfaces.

For the present, and until more material is available, no definite names are assigned to the single specimen from Honduras and the remaining collection from Guatemala, both of which are here suggested to be of possible hybrid origin between *B. guatemalensis* and the several other species probably present, but as yet uncollected, in the region. Further exploration, not only in Central America but also in the botanically little known Sierra Madre del Sur of Oaxaca and Chiapas, will be needed to shed light on the early northward dispersal of *Befaria* in the Americas as well as furnish us with additional clues useful in unraveling the tangled pattern of speciation within the genus.

KEY TO THE KNOWN MEXICAN AND CENTRAL AMERICAN SPECIES OF BEFARIA

Plants essentially glabrous.
- Flowers white.
  - Leaves glaucous below, petals 3–4.5 cm. long ..... *B. Hintonii.*
  - Leaves pale, but not glaucous below; petals 2–2.5 cm. long ..... *B. guatemalensis.*
- Flowers pink or red, petals 1–1.5–(2) cm. long ..... *B. glauca.*

Plants with a glandular pubescence.
- Upper part of inflorescence and calyces densely pubescent.
  - Flowers white, petals 2–4 cm. long ..... *B. mexicana.*
  - Flowers pink or red, petals 1–2.5 cm. long ..... × *B. discolor.*
- Upper part of inflorescence and calyces sparsely pubescent or glabrous, if glabrous, then the lower part of the inflorescence or branches sparsely pubescent; flowers predominately red or pink, or occasionally white, petals 1–2.5 cm. long ..... × *B. discolor.*

THE NEW YORK BOTANICAL GARDEN
NEW YORK, NEW YORK

THE BIOLOGY OF POLYPORUS BASILARIS

HAROLD E. BAILEY

(WITH FOUR FIGURES)

A brown pocket rot of *Cupressus macrocarpa* Hartweg has been known in California for a long time. Sporophores associated with this rot have been reported in Golden Gate Park, San Francisco, by workers in the Division of Forest Pathology, of the Bureau of Plant Industry, but, so far as the writer is aware, no detailed study nor description has been reported. The first material preserved for study was collected by Mr. H. E. Parks in 1923 on the Berkeley Campus of the University of California. Somewhat later, additional material from the same location was collected by Dr. Lee Bonar. These specimens, with study notes, were sent by Dr. Bonar to Professor L. O. Overholts, who made a study of the material. Having concluded that the fungus was an undescribed species, Overholts (in litt.) has proposed the name, *Polyporus basilaris* sp. nov. and characterized it as follows:

Polyporus basilaris Overholts sp. nov. Sporophora annua vel incremento marginale revivescens sessilis vel in substrata decurrens, crebro nodulosa in sulcis trunci leviter evoluta, quandocunque valide evoluta imbricata, tenax coriacea in sicco rigida dura; pileus applantus vel paullo convexus, 1–4 cm. longus, 2.5–6 cm. diametro 0.4–0.8 cm. crassus, sordido-griseus in sicco fumeus vel nigrescens, pubescens, pilis brevibus velutinis, mox glabratus vel fibrilloso-striatus haud incrustatus uni-vel bizonatus, zonis latis; contextum album tenax in sicco durum zonatum crassitudine 1–5 mm.; pori albi in sicco immutati vel isabellini vel hepatici, tubulis 1–3 mm. longis, oribus circularibus mox angularibus ad irregularibus integris, circa 3–4 pro mm., parietibus crassis; sporidia ellipsoidea vel angusto-ellipsoidea levia hyalina, 4–5 μ longa, 2.5–3.5 μ diametro; basidia 4–5 μ diametro; cystidia nulla; contexti hyphae hyalinae flexuosae pauci-ramosae, parietibus inconspicue incrassatis, 3–7 μ diametro, interdum conspicue nodoso-septatae.

Hab. ad basim truncorum *Cupressi macrocarpae* Hartweg.

Sporophore annual or reviving with only marginal growth, sessile or decurrent on substratum, frequently poorly developed and nodulose in furrows on the trunk, imbricate where well developed, tough and leathery when fresh, rigid and hard on drying; pileus applanate or somewhat convex 1–4 × 2.5–6 × 0.4–0.8 cm., dirty grayish, becoming grayish brown or blackish on drying, at first with short velvety pubescence, later nearly glabrous or fibrillose-striate, not incrusted, with one or two broad zones or depressions; context white, tough, drying hard, zonate, 1–5 mm. thick; pore surface white when fresh, unchanged in drying or becoming isabelline or dirty buff, the tubes 1–3 mm. long, their mouths circular then subangular to irregular, thick-walled, entire, averaging 3–4 per mm.; spores ellipsoid or narrow ellipsoid, smooth, hyaline, 4–5 × 2.5–3.5 μ; basidia 4–5 μ diam.; cystidia none; context hyphae hyaline, flexuous, sparingly branched, the walls not conspicuously thickened, 3–7 μ diam., with conspicuous though not abundant clamps.

On the lower portion of the trunks *Cupressus macrocarpa* Hartweg.

Type collected at Berkeley, California, Feb. 14, 1923 by (H. E. Parks Herb. 1431; Overholts Herb. 8527); also collected in the same locality and on the same host by Lee Bonar.

"Mr. Bonar has contributed largely to the description here presented. The species has the aspect of a trametoid and over-developed *Polyporus versicolor*, but the spores are very different. From *Fomes annosus* it differs in the less globose spores, in the clamped context hyphae, and in being associated with a very different rot."

Monterey Cypress (*Cupressus macrocarpa* Hartw.), the host of *P. basilaris*, is a narrow endemic in the vicinity of Monterey, California. It occurs indigenously only on the coastal bluffs seldom more than a half mile inland in the region of Monterey. The tree, in spite of its very restricted natural habitat, is readily propagated and has been extensively planted as an ornamental, particularly in California. Certain facts: viz., that the sporophore is a small inconspicuous annual appearing only during moist weather, that the host is not widely distributed, and that the sporophores are relatively rare, are largely responsible for the obscurity of the fungus. In addition, the sporophore usually arises in the natural crevices and convolutions of the trunk, dries out rapidly, and changes color from a smoky-gray to a brown color which blends with the color of the bark and makes detection difficult. Insects frequently attack the sporophores and reduce them in a few weeks to an unrecognizable form.

The stands of *C. macrocarpa* from which data for this paper were obtained were as follows: (1) in the vicinity of the Greek Theatre on the University of California campus at Berkeley, trees planted in 1867; (2) in Sequoia Park in the Oakland Hills, at an elevation 800 feet higher than stand (1); (3) in Golden Gate Park, San Francisco, California; (4) in the vicinty of Monterey, California.

Because of a blight, caused by *Coryneum* sp., and a bark beetle, *Phloeosinus cupressi* Hopk., which had infected the cypress, many of the trees in these areas were being cut to check further infection. This offered an opportunity to make observations on freshly sectioned trunks. A pocket rot was found to be common in all of the older trees in the above-mentioned

TABLE 1

Number of trees in four stands of C. macrocarpa containing pocket rot associated with Polyporus basilaris.

Location	No. of trees	Trees with rot	Percentage with rot	Average age
U. C. Campus	78	70	88.5	65 years
Sequoia Park	349	5	1.43	26 "
Golden Gate Park	81	15	18.5	34 "
Monterey	83	68	82.0	No estimate

stands. Data were obtained on the diameter of the trunks and the amount of rot shown in cross section at stump height, approximately 2 feet above ground.

More detailed studies were made on trees in the campus stand. These included sketches of cross and longitudinal sections of the base of the trunk and estimates of the area of decay, the longitudinal extent of the rot, and the presence or absence of the rot in the roots. Trunks of two trees were split longitudinally into quarters and the sections sketched and measured in order to compare the actual amount of sound and decayed wood. It was found from this more detailed study of 71 trees that 32 per cent contained only a small amount of decay ($\frac{1}{4}$ to 5 square inches as shown in cross-section). However, all the trees over three feet in diameter showed infection, and 60 per cent of these contained over 50 square inches of decay. All cross-section measurements were made at stump height.

The pocket rot so commonly found in the trunk of the tree is found less commonly in the roots. In the larger roots it may penetrate a few inches but generally not to any great depth. On the campus it was possible to make a study of the root system because the trees were pulled down and their roots thus exposed. While 20 per cent of the trees were found to contain occasional pockets of rot in their roots, badly infected trees seldom showed more than two or three small pockets in the entire root system.

Cross and longitudinal sections of the cypress trunks show that the infection of the tree ordinarily takes place in the lower part of the trunk. In young trees, in which the rot was in the incipient stages, the infection was without exception present in the lower portion of the tree. The height of the rot as determined from an examination of thirty-two trees was as follows: twenty-six of the trees were found to have the rot limited to less than twelve feet above the ground line and only five exceeded this height. The highest extension of the rot found in the trunk of any cypress was seventeen feet. In this tree it was found to be present in the larger branches.

In trees showing the beginning stages of decay a cross-section of the trunk often has but a single pencil of rot. When sections of such trees are split longitudinally, these pencils may be traced for a distance of a few inches to two feet, depending upon the degree of development of the rot. The infected wood in the incipient stage is almost normal in appearance, but usually tinged with yellow. These infected pockets, upon drying out, become darker in color and show fine checks. In badly decayed trees one or more pencils of rot may be found on the advancing line of decay. The decay does not proceed into uninfected areas as an ever increasing spherical mass of rot, but rather spreads by means of long finger-like protrusions into the heartwood. As the rot proceeds, it becomes very complete, leaving a brown crumbly residue which cracks into square or rectanglar blocks. In the advanced stages

of decay the pockets coalesce so that they form masses of rot with thin irregular strips of firm wood scattered through the mass. Wefts of white mycelium frequently appear in the cracks of the rot. There is no apparent discoloration in the wood beyond the sharp border of the pockets.

Microscopic examination of the decayed wood showed that the hyphae were rare in the pockets of rot. However, they are found abundantly in a zone about six millimeters wide between the border of the pocket of rot and the sound wood. The hyaline hyphae are 0.7–1.5 μ in diameter and branch freely as they pass from one cell to another. The penetration of the cell walls may be through bordered pits or directly through the wall. Tissue cultures made from the edges of the pockets and from the incipient stages of decay bear out the results obtained from the microscopical studies, i.e., that active mycelium is found only in a narrow margin around the pocket of rot. Negative results in culture tests were obtained from wood beyond 6 mm. from the border of the pockets and from the sound wood between the pockets. Extremely decayed wood either failed to produced any fungus in culture, or, as was generally the case, was contaminated with other fungi, such as various species of *Penicillium* and *Trichoderma*.

Three other basidiomycetes have been reported on *Cupressus macrocarpa*:[1] *Polyporus carbonarius* Murrill, *Polyporus cutifractus* Murrill, and *Hydnum ochraceum* (Pers.) Fries. However, during this investigation no fruiting bodies other than those of *P. basilaris* have been collected from Monterey cypress.

Figure 1 shows isolated pockets as they appear in cross section; figure 2 shows a longitudinal section from the bole of the tree with pockets of rot extending upward from the generally rotted base. This condition of the base probably arose from the single pocket which increased in size and at the same time formed branch pockets.

Every sporophore collected has been found to arise from the main part of the trunk, usually in the vicinity of decayed knots. If the point of origin of the sporophore is any indication of the original point of infection, as it is considered to be in some instances, this would be added proof that infection occurs through the base of the tree.

One or more sporophores may develop on a single cypress tree and as many as four have been collected from a single trunk during the same season. Fruiting bodies have been collected on old stumps as well as on trunks of living trees. Longitudinal sections of the trunk cut at the point of infection often show the rotted wood in the vicinity of the sporophore to be thoroughly moist. The presence of moisture is probably important in the development of the sporophores. In the Berkeley region they appear during the rainy season, usually in the latter part of November or the early part of

[1] Seymour, A. B., Host index of the fungi of North America, p. 72. 1929.

December. From then on they may continue to develop until the middle of February, or later, although the majority of sporophores have usually passed their prime and many have ceased to sporulate by that time.

The sporophores first appear as nodular fleshy sheets of grayish tissue. From these foundations shelves appear, growing from the upper marginal part of the sporophore. Frequently one finds cavities which are completely shut off from the outside and yet contain well developed spore-producing tubes inside. These cavities are particularly common in the older parts of sporophores where there has been a growing over or coalescing of one shelf of the sporophore with another. Sporophores have been found to recur season after season on the same trees and in the same places on the trunk. Mature sporophores vary greatly in type, size, and shape. The imbricate shelving type is the most commonly found (figure 3), but one finds also sporophores which are resupinate, nodulose, or even fleshy "sparassus" types.

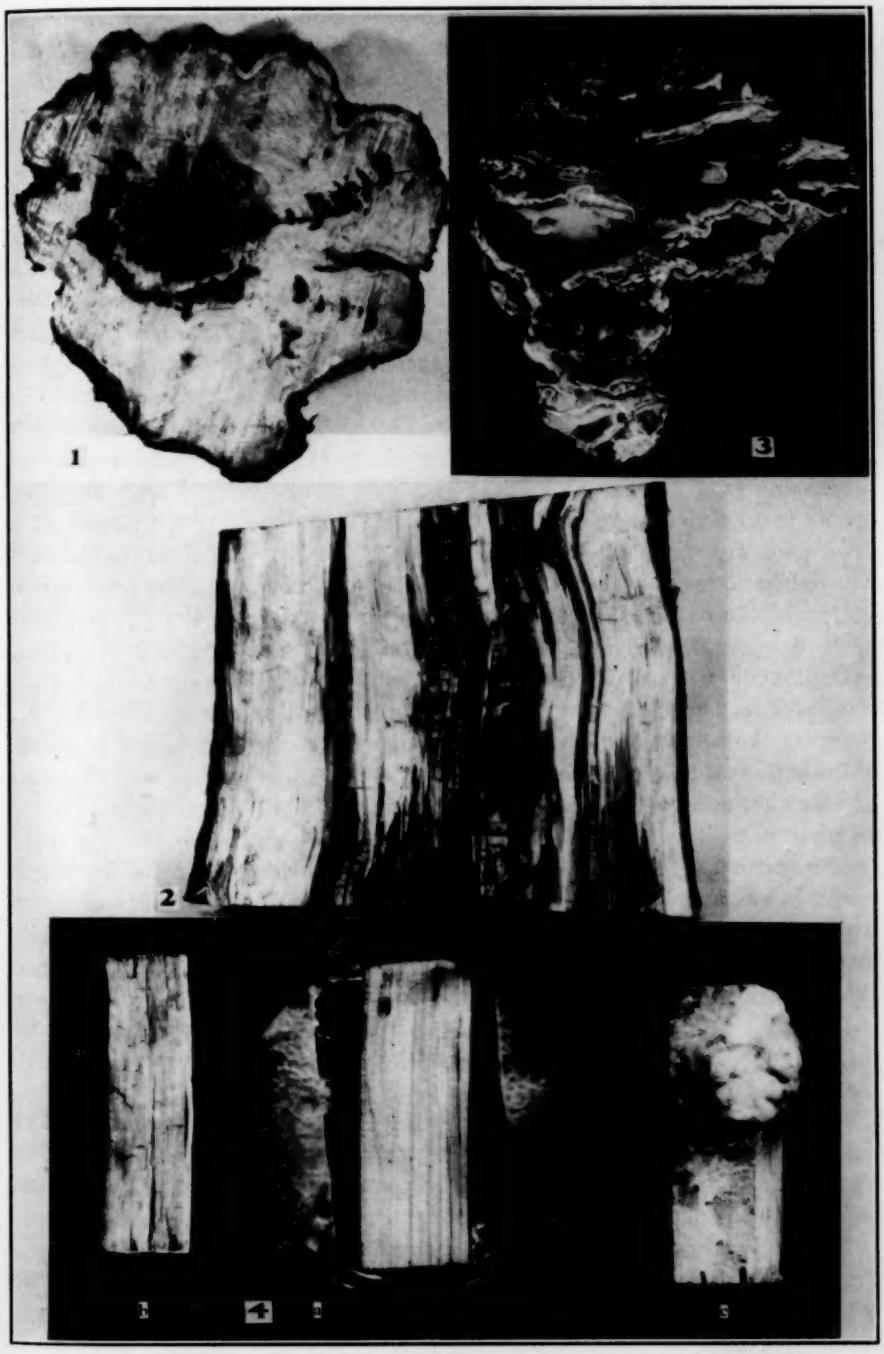
Tissue cultures from the sporophores of *P. basilaris* frequently produce Poria-like fruiting bodies on agar slants and in Petri dishes (figure 4). If the test tube or the Petri dish is inverted so that the spores may collect on the lid, a heavy coating of spores is deposited on the glass. Abortive sporophores may also develop on blocks of wood. The under surface of such sporophores is composed of lamella-like rays. Sporulation has not, however, been observed to occur from these fruiting bodies.

Sporulation in nature may continue over a period of several weeks. Daily observations have showed that some of the fruiting bodies may shed spores continuously for a month and a half. The average period of sporulation, however, is about half of this time. From large sporophores sporulation is often so heavy that during an interval of twenty-four hours a glass slide placed underneath will have a heavy white coating.

Spores of *Polyporus basilaris* have a very low percentage of germination in culture. Samples for the germination studies were obtained from the field on sterile glass slides by the use of spore traps, or, in some cases the whole sporophore was collected and allowed to shed spores directly upon the medium. Spores were gathered from different sporophores. Substrata of various compositions and pH were used, but negative results in the spore germination studies were obtained except with one medium, containing

Explanation of figures 1–4.

FIG. 1. Cross-section through trunk of *Cupressus macrocarpa* showing isolated pockets of rot outside the generally rotted center. FIG. 2. Longitudinal section through lower portion of trunk of *Cupressus macrocarpa* showing pockets of rot extending upwards from a generally rotted base. FIG. 3. Sporophore of *Polyporus basilaris*, typical imbricate type. FIG. 4. *a*, Surface view of Petri dish containing a block of wood of *Cupressus macrocarpa* on a glass plate over a young culture of *Polyporus basilaris*; *b*, rotted wood block after exposure to the fungus 120 days; *c*, sporophore of *Polyporus basilaris* developing on wood block in culture.


1
3
2

1 per cent peptone, 1 per cent glucose, and 2 per cent agar, with a few shavings of *C. macrocarpa*. The spores from a spore suspension were dispersed on the substrate with a sterile platinum loop. An examination of the plates two days after inoculation showed that approximately 50 per cent of the spores had germinated. Later attempts with the same medium did not prove successful.

In order to study the effect of the rot on the wood, artificial inoculation was undertaken in the laboratory. The procedure used in preparing sound wood for inoculation was as follows: A section of trunk 16 inches in diameter was sawed into blocks one inch square by three inches long. These were soaked in water under a bell jar for about an hour at reduced pressure. They were then sterilized in an Arnold sterilizer on three successive days for periods of a half hour. At the conclusion of this treatment a moisture content of 40–50 per cent was obtained, as determined by weighing samples, then drying out these samples and reweighing. The blocks of wood were then removed aseptically from the containers in which they were sterilized and placed on sterile glass slides in specially prepared culture dishes; these contained a growth of mycelium of *P. basilaris*, with which they had been inoculated. The culture dishes had been prepared some days previous to the preparation of the blocks of wood, so that the fungus isolated from the tissue of the sporophore could develop a heavy mat of mycelium. Two per cent malt extract agar was used as a culture medium. The dishes containing the blocks of wood were then placed in a humid chamber at 25° C $\pm$ 3°. Starting the wood to rot over a young vigorous mat of mycelium considerably shortens the time required to rot the wood. By this method several points of infection occur at once and the fungus is able to maintain itself on the nutrient until it becomes established on the wood.

The growth of the mycelium of *P. basilaris* over the blocks of *C. macrocarpa* in culture is rather slow. Only a thin threadlike reticulum of interwoven hyphae covers the surface of the block of wood in a sparse white growth. A block of wood split lengthwise with the grain, however, shows that the fungus penetrates the wood readily. A thin white coating of mycelium can sometimes be observed on the freshly split surface. The wood seems waxy to the touch. In the last stages of decay it is brittle, and when it dries out many fine cracks develop (fig. 4*b*).

Chemical analyses were made at three periods during the process of decay so that various changes in the composition of the wood could be noted. Preparation for the analyses was as follows: Wood blocks which had been exposed to the activity of the fungus for a certain period were taken from culture, dried, and the loss in weight determined. The rotted wood was then ground, sieved, and divided into 60–80 mesh and 80–100 mesh samples. The sawdust used in the sound wood analysis was prepared by holding a rasp

against a block of wood as it was turned on a lathe. The method of the Forest Products Laboratory[2] was used in the analysis of the wood samples except as follows: the water soluble component was determined by extracting, with the use of a Soxlet extractor, for four hours at a temperature of 85° C. The chlorine gas was only approximately measured and no cooling system was used around the chlorinating chamber. Although these changes in procedure are not conventional, any method of wood analysis now available is subject to criticism. The results showed, in general, that there was a progressive utilization of wood components in the wood. Samples taken at successive intervals during exposure to the fungus showed losses in weight of 10.2, 18.1, and 39 per cent. Table 2 shows the relative percentages of the components of the rotted wood, sound wood, and sporophore tissue. The values obtained from the sporophore tissue differ in several respects from those of the sound and decayed wood.

TABLE 2

Analysis of sound and decayed wood of Cupressus macrocarpa and sporophore tissue of Polyporus basilaris.

	Wood decayed in culture			Sound wood	Sporophore
Loss in weight	10.2%	18.1%	39.0%		
Water soluble	10.68	10.19	11.50	11.05	30.14
Alcohol benzene soluble	6.91	4.18	3.78	9.77	16.0
Cellulose	40.94	32.83	18.48	46.2	43.4
Lignin	17.87	30.8	22.8	31.1	13.35
Pentosans	12.78	10.57	6.53	8.62	26.9
Ash	0.476	0.516	0.27	0.339	.....

SUMMARY

(1) A study made in the field on the occurrence of the pocket rot caused by *P. basilaris* in *C. macrocarpa* showed that only 2 per cent of the trees were infected by the time they were 26 years old, while in another stand 88 per cent of the trees were infected at the average age of 65 years.

(2) The rot caused by *P. basilaris* was found to be limited to the bole of the tree. Only occasionally was it present in the branches and roots.

(3) In the incipient stages of decay only small pockets of rot were found. These grew in size and finally coalesced to form large masses of decayed wood.

(4) Sporophores recurring season after season have been found on both living and dead cypress trunks. Sporulation from these was found to occur for periods up to 45 days. Sporulating fruiting bodies were obtained in culture and spore germination secured.

(5) *P. basilaris* was grown in culture on specially prepared wood blocks of *C. macrocarpa* and the rate of growth found to be very slow.

[2] Bray, M. W., in Paper Trade Jour. 87(25): 59–68. 1928.

(6) Analyses made on wood samples from blocks which had lost 10.2, 18.1, and 39 per cent weight during the process of decay showed a progressive utilization by the fungus of many of the components of the wood.

The author is greatly indebted to Dr. Lee Bonar, Department of Botany, University of California, who began the original investigation on *Polyporus basilaris*. His helpful advice and constant encouragement have been much appreciated.

BERKELEY, CALIFORNIA

NEW COMBINATIONS AND NEW NAMES IN THE UMBELLIFERAE

MILDRED E. MATHIAS AND LINCOLN CONSTANCE

The authors have been preparing a revision of the Umbelliferae for North American Flora. The purpose of this paper is to list certain new combinations and new names.

ARRACACIA Baner. in Jamaica Jour. **4**: 18. 1826. The following new combinations and new names are necessary:

Arracacia arguta (Rose) Mathias & Constance, comb. nov. *Museniopsis arguta* Rose, Contr. U. S. Nat. Herb. **8**: 336. 1905. *Tauschia drudeophytoides* Macbr., Contr. Gray Herb. n. s. **56**: 33. 1918.

ARRACACIA ATROPURPUREA (Lehm.) Benth. & Hook. var. **brevipes** (Coult. & Rose) Mathias & Constance, comb. nov. *Arracacia brevipes* Coult. & Rose, Contr. U. S. Nat. Herb. **3**: 296. 1895.

Arracacia anomala Mathias & Constance, nom. nov. *Museniopsis pubescens* Coult. & Rose, Proc. Wash. Acad. **1**: 134. 1900. Not *Arracacia pubescens* Wolff, 1910. *Tauschia pubescens* Macbr. Contr. Gray Herb. n. s. **56**: 33. 1918.

ARRACACIA TOLUCENSIS (HBK) Hemsl. var. **multifida** (Wats.) Mathias & Constance, comb. nov. *Arracacia multifida* Wats. Proc. Amer. Acad. **26**: 136. 1891. *Arracacia dissecta* Coult. & Rose, Proc. Wash. Acad. **1**: 141. 1900. *Arracacia Dugesii* Coult. & Rose, op. cit., p. 141. *Arenaria multifida* Wats. ex Durand & Jackson, Ind. Kew Suppl. **1**: 36. 1902, error. *Arracacia tenuifolia* Rose, Contr. U. S. Nat. Herb. **10**: 127. 1906.

Arracacia Schiedei (Wolff) Mathias & Constance, comb. nov. *Nematosciadium Schiedei* Wolff in Fedde, Repert. **9**: 419. 1911.

TAUSCHIA Schlecht. Linnaea **9**: 607. 1834. Not *Tauschia* Preissler, 1828. *Deweya* Torr. & Gray, Fl. N. Amer. **1**: 641. 1840. *Museniopsis* Coult. & Rose, Rev. N. Amer. Umbel. 26, 122. 1888, as to type species only. *Hesperogenia* Coult & Rose, Contr. U. S. Nat. Herb. **5**: 203. 1899. *Drudeophytum* Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 80. 1900. *Valaea* DC. of authors in part.

Macbride[1] was correct in referring these various species to the one genus *Tauschia* Schlecht. which has been conserved over *Tauschia* Preissler. The following new combinations are necessary:

Tauschia glauca (Coult. & Rose) Mathias & Constance, comb. nov. *Velaea glauca* Coult. & Rose, Contr. U. S. Nat. Herb. **3**: 321, *pl. 14*. 1895. *Drudeophytum glaucum* Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 82. 1900. *Veleae glauca* var. *purpurascens* J. T. Howell, Leafl. West. Bot. **2**: 185. 1939.

Tauschia Stricklandi (Coult. & Rose) Mathias & Constance, comb. nov. *Hesperogenia Stricklandi* Coult. & Rose, Contr. U. S. Nat. Herb. **5**: 203, *pl. 27*. 1899. *Zizia Stricklandi* K.–Pol. Bull. Soc. Nat. Mosc. n. s. **29** (1915): 200. 1916.

---

[1] Macbride, Contr. Grey Herb. n. s. **56**: 28. 1918.

Tauschia tenuifolia (Wats.) Mathias & Constance, comb. nov. *Eulophus tenuifolius* Wats. Proc. Amer. Acad. **23**: 276. 1888. *Velaea tenuifolia* Drude in Engl. & Prantl, Pflanzenfam. **3** (8): 169. 1898. *Museniopsis tenuifolia* Coult. & Rose, Contr. U. S. Nat. Herb. **3**: 302. 1895.

DONNELLSMITHIA Coult. & Rose, Bot. Gaz. **15**: 15. 1890. *Museniopsis* Coult. & Rose, Rev. N. Amer. Umbel. 26, 122. 1888, as to Mexican species, not as to type species. *Schiedeophytum* Wolff, in Fedde Repert. **9**: 419. 1911.

The genus *Donnellsmithia*, based on the species *D. guatemalensis*, has been considered monotypic. This study has shown that the numerous Mexican species referred to *Museniopsis* and the monotypic genera *Donnellsmithia* and *Schiedeophytum* constitute a natural generic unit for which the name *Donnellsmithia* must be used. The following new combinations are necessary.

Donnellsmithia biennis (Coult. & Rose) Mathias & Constance, comb. nov. *Museniopsis aegopodioides* Coult. & Rose, Contr. U. S. Nat. Herb. **3**: 302. 1895, as to specimens cited, not as to synonymy and not including name-bearing synonym. *Museniopsis biennis* Coult. & Rose, Proc. Wash. Acad. **1**: 130. 1900. *Tauschia biennis* Macbr. Contr. Gray Herb. n. s. **56**: 32. 1918.

Donnellsmithia cordata (Coult. & Rose) Mathias & Constance, comb. nov. *Museniopsis cordata* Coult. & Rose, Contr. U. S. Nat. Herb. **3**: 304. 1895. *Museniopsis scabrella* Coult. & Rose, op. cit., p. 304. *Velaea cordata* Drude in Engl. & Prantl, Pflanzenfam. **3** (8): 169. 1898. *Velaea scabrella* Drude, op. cit., p. 169. *Tauschia scabrella* Macbr. Contr. Gray Herb. n. s. **56**: 33. 1918.

Donnellsmithia dissecta (Coult. & Rose) Mathias & Constance, comb. nov. *Museniopsis dissecta* Coult. & Rose, Contr. U. S. Nat. Herb. **3**: 304. 1895. *Velaea dissecta* Drude in Engl. & Prantl, Pflanzenfam. **3** (8): 169. 1898. *Tauschia pinetorum* Brandegee, Univ. Calif. Publ. Bot. **10**: 413. 1924.

Donnellsmithia madrensis (Coult. & Rose) Mathias & Constance, comb. nov. *Museniopsis madrensis* Coult. & Rose, Proc. Wash. Acad. **1**: 130. 1900.

Donnellsmithia mexicana (Robins.) Mathias & Constance, comb. nov. *Pimpinella mexicana* Robins. Proc. Amer. Acad. **26**: 164. 1891. *Schiedeophytum fallax* Wolff in Fedde, Repert. **9**: 420. 1911. *Schiedeophytum mexicanum* Wolff in Engl. & Prantl, Pflanzenr. **90**: 326. 1927.

Donnellsmithia ovata (Coult. & Rose) Mathias & Constance, comb. nov. *Museniopsis ovata* Coult. & Rose, Proc. Wash. Acad. **1**: 133. 1900. *? Museniopsis biennis* var. *pinnatisecta* Riley, Kew Bull. **1924**: 22. 1924.

Donnellsmithia peucedanoides (HBK) Mathias & Constance, comb. nov. *Cnidium peucedanoides* HBK. Nov. Gen. et Sp. **5**: 15. 1821. *Eulophus peucedanoides* Benth. & Hook. Gen. Pl. **1**: 885. 1867. *Eulophus ternatus* Wats. Proc. Amer. Acad. **23**: 276. 1888. *Museniopsis peucedanoides* Coult. & Rose, Contr. U. S. Nat. Herb. **3**: 303. 1895. *Museniopsis ternata* Coult. & Rose, op. cit., p. 303. *Museniopsis ternata* var. *filifolia* Coult. & Rose, op. cit. p. 303. *Museniopsis Schaffneri* Coult. & Rose, op. cit., p. 303. *Velaea peucedanoides* Drude in Engl. & Prantl, Pflanzenfam. **3** (8): 169. 1898. *Velaea ternata* Drude, op. cit., p. 169. *Velaea Schaffneri* Drude, op. cit., p. 169. *Tauschia peucedanoides* Macbr. Contr. Gray Herb. n. s. **56**: 32. 1918. *? Peucedanum junceum* Humb. & Bonpl. ex Spreng. in Roem. & Schult. Syst. Veg. **6**: 572. 1820.

DONNELLSMITHIA PEUCEDANOIDES (HBK) Mathias & Constance var. **purpurea** (Coult. & Rose) Mathias & Constance, comb. nov. *Museniopsis purpurea* Coult. & Rose, Proc. Wash. Acad. **1**: 132. 1900.

Donnellsmithia reticulata (Coult. & Rose) Mathias & Constance, comb. nov. *Museniopsis reticulata* Coult. & Rose, Proc. Wash. Acad. **1**: 133. 1900.

Donnellsmithia serrata (Coult. & Rose) Mathias & Constance, comb. nov. *Museniopsis serrata* Coult. & Rose, Contr. U. S. Nat. Herb. **3**: 304. 1895. *Velaea serrata* Drude in Engl. & Prantl, Pflanzenfam. **3** (8): 170. 1898.

Donnellsmithia submontana (Coult. & Rose) Mathias & Constance, comb. nov. *Museniopsis submontana* Coult. & Rose, Proc. Wash. Acad. **1**: 131. 1900.

Donnellsmithia tuberosa (Coult. & Rose) Mathias & Constance, comb. nov. *Museniopsis tuberosa* Coult. & Rose, Contr. U. S. Nat. Herb. **3**: 303. 1895. *Velaea tuberosa* Drude in Engl. & Prantl, Pflanzenfam. **3** (8): 169. 1898. *Museniopsis tenuissima* Coult. & Rose, Proc. Wash. Acad. **1**: 131. 1900. *Museniopsis glauca* Coult. & Rose, op. cit., p. 131. *Museniopsis fusiformis* Rose, Contr. U. S. Nat. Herb. **8**: 337. 1905. *Tauschia fusiformis* Macbr. Contr. Gray Herb. n. s. **56**: 32. 1918.

LEPTOTAENIA Nutt.

Leptotaenia Hendersoni (Coult. & Rose) Mathias & Constance, comb. nov. *Peucedanum Hendersoni* Coult. & Rose, Bot. Gaz. **13**: 210. 1888; Rev. N. Amer. Umbel. 56. 1888. *Leptotaenia Leibergi* Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 202, *pl. 7*. 1900. *Lomatium Hendersoni* Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 209. 1900. *Cogswellia Hendersoni* Jones, Contr. Western Bot. **12**: 33. 1908.

Since the publication of the revision of the genus *Lomatium*[1] the authors have seen specimens of the type collection of *Peucedanum Hendersoni* deposited in the herbarium of the University of Oregon. This species is without question identical with *Leptotaenia Leibergi*.

LIGUSTICELLA Coult. & Rose

Ligusticella Macounii (Coult. & Rose) Mathias & Constance, comb. nov. *Ligusticum Macounii* Coult. & Rose, Contr. U. S. Nat. Herb. **1**: 289. *pl. 23*. 1893. *Orumbella Macounii* Coult. & Rose, Contr. U. S. Nat. Herb. **12**: 446. 1909.

LIGUSTICUM L.

LIGUSTICUM FILICINUM Wats. var. **tenuifolium** (Wats.) Mathias & Constance, comb. nov. *Ligusticum tenuifolium* Wats. Proc. Amer. Acad. **14**: 293. 1879. *Ligusticum oreganum* Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 138. 1900.

LIGUSTICUM PORTERI Coult. & Rose var. **brevilobum** (Rydb.) Mathias & Constance, comb. nov. *Ligusticum brevilobum* Rydb. Fl. Rocky Mts. 613, 1064. 1917.

POLYTAENIA DC.

Polytaenia texana (Coult. & Rose) Mathias & Constance, comb. nov. *Polytaenia Nuttallii* var. *texana* Coult. & Rose, Contr. U. S. Nat. Herb. **7**:

[1] Mathias, Ann. Mo. Bot. Gard. **25**: 225–297. 1938.

192. 1900. *Pleiotaenia Nuttallii* var. *texana* Coult. & Rose, op. cit. **12**: 448. 1909. *Phanerotaenia texana* St. John, Rhodora **21**: 182. 1919.

RHODOSCIADIUM Coult. & Rose.

The genus *Deanea* Coulter and Rose is referred to *Rhodosciadium*, making the following new combinations and new names necessary:

Rhodosciadium argutum (Rose) Mathias & Constance, comb. nov. *Deanea arguta* Rose, Contr. U. S. Nat. Herb. **10**: 128. 1906.

Rhodosciadium diffusum (Coult. & Rose) Mathias & Constance, comb. nov. *Deanea diffusa* Coult. & Rose, Proc. Wash. Acad. **1**: 155. 1900.

Rhodosciadium longipes (Rose) Mathias & Constance, comb. nov. *Deanea longipes* Rose, Contr. U. S. Nat. Herb. **10**: 128. 1906.

Rhodosciadium macrophyllum Mathias & Constance, nom. nov. *Deanea glauca* Coult. & Rose, Proc. Wash. Acad. **1**: 156. 1900. Not *Rhodosciadium glaucum* Coult. & Rose. 1895.

Rhodosciadium montanum (Coult. & Rose) Mathias & Constance, comb. nov. *Deanea montana* Coult. & Rose, Proc. Wash. Acad. **1**: 155. 1900.

Rhodosciadium Nelsoni (Coult. & Rose) Mathias & Constance, comb. nov. *Deanea Nelsoni* Coult. & Rose, Proc. Wash. Acad. **1**: 155. 1900.

Rhodosciadium purpureum (Rose) Mathias & Constance, comb. nov. *Deanea Pringlei* Rose, Contr. U. S. Nat. Herb. **10**: 128. 1906. Not *Rhodosciadium* Pringlei Wats., 1890. *Deanea purpurea* Rose, op. cit., p. 128.

SPERMOLEPIS Raf.

Spermolepis inermis (Nutt.) Mathias & Constance, comb. nov. *Leptocaulis inermis* Nutt. ex DC. Coll. Mem. **5**: 39, *pl. 10, f. b.* 1829. *Leptocaulis patens* Nutt. ex DC. Prodr. **4**: 107. 1830. *Apium patens* Wats. Bibl. Ind. **1**: 413. 1878. *Apiastrum patens* Coult. & Rose, Rev. N. Amer. Umbel. 110. 1888. *Spermolepis patens* Robins. Rhodora **10**: 34. 1908. *Spermolepis patens* var. *inermis* Mathias, Brittonia **2**: 243. 1936.

DEPARTMENT OF BOTANY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

INDEX TO AMERICAN BOTANICAL LITERATURE

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Papers that relate exclusively to bacteriology, forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

This Index is reprinted monthly on cards, and furnished in this form to subscribers at the rate of three cents for each card. Selections of cards are not permitted; each subscriber must take all cards published during the term of his subscription. Correspondence relating to the card issue should be addressed to the Treasurer of the Torrey Botanical Club.

Albaum, H. G., Kaiser, S. & Eichel, B. Coleoptile growth after preliminary soaking of oat grains in various solutions. Am. Jour. Bot. **27**: 619–623. *f. 1–5.* 12 N 1940.

Alexander, E. J. *Meiracyllium trinasutum* refound. Bull. Am. Orchid Soc. **9**: 144. *illust.* N 1940.

Alexander, E. J. Southern plant notes. Castanea **5**: 91–93. O 1940.

Allard, H. A. *Phacelia ranunculacea* (Nutt.) Constance, its length of day, temperature reactions and seasonal adaptations. Castanea **5**: 94–97. O 1940.

Ames, O. A new species of *Pseudacoridium*. Bull. Am. Orchid Soc. **9**: 45. *pl. 44.* Jl 1940.

Andrews, A. L. Bryaceae (II) Mniaceae, Rhizogoniaceae *in* Grout, A. J. Moss Flora of North America north of Mexico. **2**[1]: 211–262. *pl. 84–109.* S 1940.

Axelrod, D. I. A record of *Lyonothamnus* in Death Valley, California. Jour. Geol. **48**: 526–531. *f. 1.* Jl–Au 1940.

Backus, E. J. & Keitt, G. W. Some nuclear phenomena in *Venturia inaequalis*. Bull. Torrey Club **67**: 765–770. *f. 1–22.* 3 D 1940.

Barton, L. V. Toxicity of ammonia, chlorine, hydrogen cyanide, hydrogen sulphide, and sulphur dioxide gases. IV. Seeds. Contr. Boyce Thompson Inst. **11**: 357–363. O–D 1940.

Beams, H. W., Zell, L. W. & Sulkin, N. M. Cytological studies on yeast cells with special reference to the budding process. Cytologia **11**: 30–36. *f. 1, 2.* O 1940.

Benson, L. The cacti of Arizona. Univ. Ariz. Biol. Sci. Bull. **4**: 1–134. *pl. 1–52.* 1 Ja 1940.

Bergner, A. D., Avery, A. G. & Blakeslee, A. F. Chromosomal deficiencies in *Datura stramonium* induced by colchicine treatment. Am. Jour. Bot. **27**: 676–683. *f. 1–3.* 12 N 1940.

Berry, E. W. A *Cusparia* from the Pliocene of trans-Andean Bolivia. Jour. Wash. Acad. Sci. **30**: 464–467. *f. 1–7.* 15 N 1940.

Blachly, C. D. A sectorial chimera in the canna. Jour. Hered. **31**: 453–454. *f. 15–16.* O 1940.

Blake, S. F. New species and new names among Arizona Asteraceae. Jour. Wash. Acad. Sci. **30**: 467–472. 15 N 1940.

Bonner, J. On the growth factor requirements of isolated roots. Am. Jour. Bot. **27**: 692–701. *f. 1.* 12 N 1940.

Brade, A. C. Contribuição para o estudo da flora Pteridophyta da serra de Baturité estado do Ceará. Rodriguesia **4**: 289–302. *pl. 1, 2.* 1940.

Bramble, W. C. & Holst, E. C. Fungi associated with *Dendroctonus frontalis* in killing shortleaf pines and their effect on conduction. Phytopathology **30**: 879–899. *f. 1–5.* N 1940.

Briley, F. (Mrs.) The effect of daylight on plant growth. 37th Ann. Rep. South Dakota Hort. Soc. 66–68. 1940.

Brooks, R. M. Comparative histogenesis of vegetative and floral apices in *Amygdalus communis* with special reference to the carpel. Hilgardia **13**: 249–299. *pl. 1–44* + *f. 1–11.* Au 1940.

Buchholz, J. T. The embryogeny of *Torreya*, with a note on *Austrotaxus*. Bull. Torrey Club **67**: 731–754. *f. 1–44.* 3 D 1940.

Burkholder, P. R. & McVeigh, I. Growth of *Phycomyces Blakesleeanus* in relation to varied environmental conditions. Am. Jour. Bot. **27**: 634–640. *f. 1–4.* 12 N 1940.

Burret, M. Um caso de hibridacao entre *Arecastrum romanzoffianum* e *Butia capitata*. Rodriguesia **4**: 277. *3 pl.* 1940.

Butler, E. T. Studies in the Patellariaceae. Mycologia **32**: 791–823. *f. 1–10.* N–D 1940.

Cash, E. K. & Davidson, R. W. Some new species of Ascomycetes on coniferous hosts. Mycologia **32**: 728–735. *f. 1–2.* N–D 1940.

Christensen, C. M. Observations on *Polyporus circinatus*. Phytopathology **30**: 957–963. *f. 1–4.* N 1940.

Cleland, R. E. Analysis of wild American races of *Oenothera* (*Onagra*). Genetics **25**: 636–644. 18 N 1940.

Coleman, L. C. The structure of homotypic and somatic chromosomes. Am. Jour. Bot. **27**: 683–686. *f. 1–8.* 12 N 1940.

Cooper, D. C. & Brink, R. A. Somatoplastic sterility as a cause of seed failure after interspecific hybridization. Genetics **25**: 593–617. *f. 1–43.* 18 N 1940.

Correll, D. S. *Epidendrum conopseum* in the southeastern states. Bull. Am. Orchid Soc. **9**: 58, 59. *1 f.* Au 1940.

Correll, D. S. Some southern orchids. Bull. Am. Orchid Soc. **9**: 79–83. S 1940.

Craig, R. T. *Mammillaria Lindsayi* sp. nov. Cactus & Succ. Jour. **12**: 182, 183. illust. N 1940.

Croizat, L. On the origin and publication of the name *Longetia*. Chron. Bot. **6**: 102. 2 D 1940.

Cuatrecasas, J. Notas a la flora de Colombia, II. Rev. Acad. Col. **12**: 425–438. *pl. 1–4* + *f. 1–24.* 31 Au 1940.

Darrah, W. C. The position of the Nematophytales. Chron. Bot. **6**: 52, 53. 4 N 1940.

Dermen, H. Colchicine polyploidy and technique. Bot. Rev. **6**: 599–635. N 1940.

Ducke, A. As espécies Brasileiras de cacau (género *Theobroma* L.), na botânica sistemática e geográfica. Rodriguesia **4**: 265–276. *7 pl.* 1940.

Earnest, E. John and William Bartram: Botanists and Explorers 1699–1777—1739–1823. i–vi, 1–187 p. Philadelphia, Univ. Penn. Press. 1940.

Eastwood, A. New western plants—II. Leafl. West. Bot. **2**: 281–285. 19 N 1940.

Eide, C. J. & Christensen, C. M. Woody decay in apple trees in Minnesota. Phytopathology **30**: 936–944. *f. 1, 2.* N 1940.

Eigsti, O. J. Methods for growing pollen tubes for physiological and cytological studies. Proc. Okla. Acad. Sci. **20**: 45–47. *f. 1–5.* 1940.

Engelbert, V. Reproduction in some *Poa* species. Canad. Jour. Res. **18**: 518–521. O 1940.

Epling, C. A new locality for *Salvia eremostachya* Jepson. Madroño **5**: 272, 273. O 1940.

Epling, C. & Robison, W. *Pinus muricata* and *Cupressus Forbesii* in Baja California. Madroño **5**: 248–250. *f. 1, 2.* 4 O 1940.

Esau, K. Developmental anatomy of the fleshy storage organ of *Daucus carota*. Hilgardia **13**: 175–209. *pl. 1–14 + f. 1–12.* Au 1940.

Esau, K. & Hewitt, W. B. Structure of end walls in differentiating vessels. Hilgardia **13**: 229–237. *pl. 1–4.* Au 1940.

Eyles, D. E. Three species of *Eleocharis* new to Georgia. Castanea **5**: 98, 99. O 1940.

Eyster, H. C. The cause of decreased germination of bean seeds soaked in water. Am. Jour. Bot. **27**: 652–659. 12 N 1940.

Fraser, W. P. Notes on the Cyperaceae of Saskatchewan. 1. *Scirpus*. Canad. Field Nat. **54**: 100, 101. O 1940.

Friesner, R. C. Key to species and varieties of ferns and fern allies of northeastern North America. Butler Univ. Bot. Stud. **4**: 142–162. Au 1940.

Friesner, R. C. An observation of the effectiveness of root pressure in the ascent of sap. Butler Univ. Bot. Studies **4**: 226, 227. Au 1940.

Friesner, R. C. Some necessary nomenclatorial changes in the genus *Solidago*. Butler Univ. Bot. Stud. **4**: 196. Au 1940.

Friesner, R. C. A study of asymmetrical growth from stump sections of *Quercus velutina*. Butler Univ. Bot. Stud. **4**: 197–206. Au 1940.

Gilbert, S. G. Evolutionary significance of ring porosity in woody Angiosperms. Bot. Gaz. **102**: 105–120. *f. 1–12.* 15 O 1940.

Giles, N. The effect of fast neutrons on the chromosomes of *Tradescantia*. Proc. Nat. Acad. Sci. **26**: 567–575. S 1940.

Godoy, E. F. El mildew o tizón del pimiento producido por la *Phytophthora capsici* en la república Argentina. Rev. Fac. Agron. Univ. Nac. La Plata **24**: 235–280. *pl. 1–7 + f. 1–3.* 1940.

Godoy, E. F. & Coste, A. D. El "mildew" del tabaco en la region tabacalera de Salta. Rev. Arg. Agron. **7**: 221–227. *f. 1–4.* 10 S 1940.

Gonçalves, C. R. Observações sobre *Pseudococcus comstocki* (Kuw., 1902) atacando citrus na baixada fluminense. Rodriguesia **4**: 179–198. *19 pl.* 1940.

Grace, Sister M. C. The genus *Psoralea*. Proc. Okla. Acad. Sci. **20**: 89–92. 1940.

Graves, A. H. Breeding work toward the development of a timber type of blight-resistant chestnut. Report for 1939. Bull. Torrey Club **67**: 773–777. 3 D 1940.

Gregory, W. C. Experimental studies on the cultivation of excised anthers in nutrient solutions. Am. Jour. Bot. **27**: 687–692. *f. 1–16.* 12 N 1940.

Groh, H. & Minshall, W. H. The ragweed situation in eastern Canada in relation to hay fever. Canad. Med. Assoc. Jour. **43**: 258–260. 1940.

Grout, A. J. List of mosses of North America north of Mexico. Bryologist **43**: 107–131. O 1940.

Grout, A. J. Moss Flora of North America north of Mexico. Appendix to Volume II. 2^{e}: 263–273. *pl. 110–115.* S 1940.

Groves, J. W. Some *Dermatea* species and their conidial stages. Mycologia **32**: 736–751. *f. 1–13.* N–D 1940.

Heller, A. A. *Lupinus* studies—I. Leafl. West. Bot. **2**: 279–281. 19 N 1940.

Hoover, R. F. Observations on California plants—I. Leafl. West. Bot. **2**: 273–278. 19 N 1940.

Hotson, H. H. The genus *Armillaria* in western Washington. Mycologia **32**: 776–790. *f. 1–3.* N–D 1940.

Howell, J. T. On the distribution of *Hulsea heterochroma.* Leafl. West. Bot. **2**: 289. 19 N 1940.

Howell, J. T. Studies in *Ceanothus*—V. Leafl. West. Bot. **2**: 285–289. 19 N 1940.

Janssen, R. E. Some fossil plant types of Illinois. Illinois State Mus. Sci. Papers **1**: i-xv, 1–124 *pl. 1–28.* 1940.

Kanouse, B. B. & Smith, A. H. Two new genera of Discomycetes from the Olympic National Forest. Mycologia **32**: 756–759. *f. 1.* N–D 1940.

Knudson, L. Viability of orchid seed. Bull. Am. Orchid Soc. **9**: 36–38. Jl 1940.

Laing, H. E. Respiration of the leaves of *Nuphar advenum* and *Typha latifolia.* Am. Jour. Bot. **27**: 583–585. *f. 1, 2.* 12 N 1940.

Larisey, M. M. Analysis of a hybrid complex between *Baptisia leucantha* and *Baptisia viridis* in Texas. Am. Jour. Bot. **27**: 624–628. *f. 1, 2.* 12 N 1940.

Levitt, J. & Siminovitch, D. The relation between frost resistance and the physical state of protoplasm. Canad. Jour. Res. **18**: 550–561. *f. 1.* N 1940.

Le Compte, S. B. Intermittently operated irrigator cones as indicators of soil-moisture condition in drying cylinder cultures of white lupines. Am. Jour. Bot. **27**: 586–595. *f. 1–3.* 12 N 1940.

Leonian, L. H. & Lilly, V. G. Studies on the nutrition of fungi. V. Factors affecting zygospore formation in *Phycomyces Blakesleeanus.* Am. Jour. Bot. **27**: 670–675. *pl. 1–4.* 12 N 1940.

Lindsey, A. A. Floral anatomy in the Gentianaceae. Am. Jour. Bot. **27**: 640–651. *f. 1–75.* 12 N 1940.

Ling, L. The histology of infection of susceptible and resistant selfed lines of rye by the rye smut fungus, *Urocystis occulta.* Phytopathology **30**: 926–935. *f. 1–4.* N 1940.

Long, W. H. & Plunkett, O. A. Studies in the Gasteromycetes. I. The genus *Dictyocephalos.* Mycologia **32**: 696–709. *f. 1–13.* N–D 1940.

Looser, G. Botanica miscelanea. V. Rev. Universitaria **25**^{a}: 31–33. 1940.

Looser, G. Las localidades de los tipos de los helechos chilenos. Rev. Universitaria **25**^{a}: 155–204. 1940.

Looser, G. Sobre algunos helechos de los Andes de las provincias de Curico y Talca. Rev. Universitaria **25**^{a}: 79–82. 1940.

Looser, G. Sobre el helechos *Pellaea ternifolia* en Chile y generalidades sobre este Genero. Rev. Universitaria **25**^{a}: 109–116. *2 pl.* 1940.

Luces Moretti, Z. Ensayo monografico sobre las especies venezolanas del genero *Chloris.* Bol. Soc. Venez. Cien. Nat. **45**: 1–10. *pl. 1–9.* 1940.

Lundell, C. L. Noteworthy Spermatophytes from Mexico & Central America. Phytologia **1**: 449–453. N 1940.

Lyon, C. J. Tree growth beside a rain gauge and thermometer. Ecology **21**: 425–437. *f. 1–3.* O 1940.

McCallan, S. E. A. & Weedon, F. R. Toxicity of ammonia, chlorine, hydrogen cyanide, hydrogen sulphide, and sulphur dioxide gases. II. Fungi and bacteria. Contr. Boyce Thompson Inst. **11**: 331–342. *f. 1–4.* O–D 1940.

McCoy, R. W. Floral organogenesis in *Frasera carolinensis.* Am. Jour. Bot. **27**: 600–609. *f. 1–22.* 12 N 1940.

MacDougall, T. & W[illiams], L. O. *Cattleya aurantiaca, C. Deckeri* and *C. Skinneri*. Bull. Am. Orchid Soc. **9**: 141, 142. *illust.* N 1940.

McVaugh, R. A revision of "*Laurentia*" and allied genera in North America. Bull. Torrey Club **67**: 778–798. 3 D 1940.

Martin, G. W. Some Heterobasidiomycetes from eastern Canada. Mycologia **32**: 683–695. *f. 1–9*. N–D 1940.

Metcalf, F. P. Eight new species of Leguminosae from southeastern China. Lingnan Sci. Jour. **19**: 549–563. *f. 1–8*. 26 O 1940.

Metcalf, F. P. Note on *Desmodium racemosum* (Thunb.) De Candolle. Lingnan Sci. Jour. **19**: 605. 26 O 1940.

Milanez, F. R. Observações sobre uma estranha doença das laranjeiras. Rodriguesia **4**: 199–263. *pl. 1–18*. 1940.

Mix, A. J. Errata in two recent papers on *Taphrina*. Univ. Kansas Sci. Bull. **26°**: 355, 356. 1 O 1940.

Moldenke, H. N. Additional Verbenaceae novelties. Phytologia **1**: 433–448. O 1940.

Moldenke, H. N. New species and varieties of *Vitex* from South America. Tropical Woods **64**: 29–40. 1 D 1940.

Moldenke, H. N. Novelties among the American Verbenaceae. Phytologia **1**: 453–480. N 1940.

Moldenke, H. N. Novelties in the Avicenniaceae and Verbenaceae. Phytologia **1**: 409–432, 433–448. O 1940.

Moldenke, H. N. Some new names in the Apocynaceae and Cornaceae and in various American groups. Rev. Sudam. Bot. **6**: 176–178 [1940].

Moldenke, H. N. Some new species and varieties of Verbenaceae. Caribbean Forester **2**: 13–17. O 1940.

Monachino, J. A note on *Sapium*. Bull. Torrey Club **67**: 771–772. 3 D 1940.

Moss, B. W. A comparative pollen analysis of two bogs within boundaries of the late Wisconsin glaciation in Indiana. Butler Univ. Bot. Stud. **4**: 207–216. Au 1940.

Mounce, I., Bier, J. E. & Nobles, M. K. A root-rot of Douglas fir caused by *Poria Weirii*. Canad. Jour. Res. **18**: 522–533. *pl. 1–4* + *f. 1–7*. O 1940.

Mousley, H. *Listera australis* Lindl. in the province of Quebec. Canad. Field Nat. **54**: 95, 96. *1 pl.* O 1940.

Mrak, E. M. & McClung, L. S. Yeasts occurring on grapes and in grape products in California. Jour. Bact. **40**: 395–407. *f. 1*. S 1940.

Murrill, W. A. Alachua County, Florida, soils and violets. Ecology **21**: 512, 513. O 1940.

Nabours, R. K. The masquerade of Lamarckism. Am. Nat. **74**: 562–566. N–D 1940.

Nearing, G. G. Guide to the lichens of New York Area—Part 6. Torreya **40**: 34–39. *pl. 10*. Mr-Ap 1940. Part 7. **40**: 110–117. *pl. 11*. Ju–Au 1940. Part 8. **40**: 198–208. *pl. 12*. N–D 1940.

Newton, M., Johnson, T., & Peturson, B. Seedling reactions of wheat varieties to stem rust and crown rust. Canad. Jour. Res. **18**: 490–512. O 1940.

Patrick, R. Some new diatoms from Brazil. Notulae Naturae **59**: 1–7. *f. 1–12*. 25 N 1940.

Patron, R. R. Descripcion de 35 variedades de trigo del pais con observaciones sobre la constancia de algunos caracteres morfologicos. Rev. Fac. Agron. Univ. Nac. La Plata **24**: 57–233. *pl. 1–7* + *f. 1–43*. 1940.

Patton, R. L. & Nebel, B. R. Preliminary observations on physiological and cytological effects of certain hydrocarbons on plant tissues. Am. Jour. Bot. **27**: 609–613. *f. 1.* N 1940.

Peck, M. E. Two new Compositae from the Wallowa mountains of Oregon. Madroño **5**: 247, 248. *f. 1, 2.* 4 O 1940.

Peebles, R. H. & Wheeler, L. C. Arizona plants: A new variety and new names and combinations. Jour. Wash. Acad. Sci. **30**: 473. 15 N 1940.

Person, L. H. & Martin, W. J. Soil rot of sweet potatoes in Louisiana. Phytopathology **30**: 913–916. *f. 1–6.* N 1940.

Pickel, D. B. Catálogo do Herbario da Escola Superior de Agricultura em Tapera (Pernambuco) (Herbarium Pickel). Bol. Mus. Nac. Rio de Janeiro **13**: 63–132. 1938.

Pittier, H. Notas dendrológicas de Venezuela. V. Bol. Soc. Venez. Cien. Nat. **41**: 63–78. 1939.

Pittier, H. Plantas venezolanas supuestas nuevas especificamente. IV. Bol. Soc. Venez. Cien. Nat. **44**: 79–95. 1940.

Raper, J. R. Sexuality in *Achlya ambisexualis*. Mycologia **32**: 710–727. *f. 1–4.* N–D 1940.

Ray, W. W. A new host for *Taphrina bacteriosperma*. Mycologia **32**: 752–753. *f. 1, 2.* N–D 1940.

Record, S. J. & Hess, R. W. American woods of the family Rutaceae. Tropical Woods **64**: 1–28. 1 D 1940.

Rigler, N. E. & Greathouse, G. A. The chemistry of resistance of plants to *Phymatrotrichum* root rot. VI. Fungicidal properties of fatty acids. Am. Jour. Bot. **27**: 701–704. *f. 1.* 12 N 1940.

Robbins, W. J. Light and the growth of excised roots of *Datura*. Bull. Torrey Club **67**: 762–764. *f. 1.* 3 D 1940.

Sanford, G. B. & Cormack, M. W. Variability in association effects of other soil fungi on the virulence of *Helminthosporium sativum* on wheat seedlings. Canad. Jour. Res. **18**: 562–565. N 1940.

Schade, A. L. & Thimann, K. V. The metabolism of the water mold, *Leptomitus lacteus*. Am. Jour. Bot. **27**: 659–670. *f. 1–9.* N 1940.

Schultes, R. E. A noteworthy collection of *Andira galeottiana*. Bot. Mus. Leafl. Harvard Univ. **8**: 200. 8 N 1940.

Schultes, R. E. Plantae Mexicanae VI. New and significant species of *Saurauia* from northeastern Oaxaca. Bot. Mus. Leafl. Harvard Univ. **8**: 189–199. 8 N 1940.

Schultes, R. E. *Sobralia macrantha* in Oaxaca. Bull. Am. Orchid Soc. **9**: 88, 89. *1 f.* S 1940.

Setchell, W. A. *Fucus cordatus* Turner. Proc. Nat. Acad. **26**: 643–651. *f. 1, 2.* N 1940.

Shirk, H. G. & Appleman, C. O. Oxygen respiration in wheat grain in relation to freezable water. Am. Jqur. Bot. **27**: 613–619. *f. 1–7.* 12 N 1940.

Shope, P. F. Colorado rusts of woody plants. Univ. Colo. Studies D **1**: 105–127. O 1940.

Standley, P. C. & Steyermark, J. A. Studies of Central American plants—II. Field Mus. Nat. Hist. Publ. Bot. **22**: 325–396. 31 O 1940.

Stout, G. L. A case of dodder (*Cuscuta subinclusa* Durand and Hilgard) on Valencia orange (*Citrus sinensis* Osbeck) in southern California. Bull. Dept. Agr. Cal. **29**: 121–124. *illust.* J–S 1940.

Strickland, J. C. The Oscillatoriaceae of Virginia. Am. Jour. Bot. **27:** 628–633. *f. 1.* 12 N 1940.

Tamayo, F. Anotaciones sobre el género *Calotropis* en Venezuela. Bol. Soc. Venez. Cien. Nat. **44:** 1–5. 1940.

Taylor-Vinje, M. Studies in *Ceratostomella montium.* Mycologia **32:** 760–775. *f. 1–30.* N–D 1940.

Tervet, I. W. Problems in the determination of physiologic races of *Ustilago avenae* and *U. levis.* Phytopathology **30:** 900–913. *f. 1–4.* N 1940.

Thompson, E. O. Morphological differences in *Taphrina caerulescens* upon different species of *Quercus.* Univ. Kansas Sci. Bull. **26**[7]: 357–366. *pl. 28, 29.* 1 O 1939.

Thornton, W. C. & Setterstrom, C. Toxicity of ammonia, chlorine, hydrogen cyanide, hydrogen sulphide, and sulphur dioxide gases. III. Green plants. Contr. Boyce Thomps. Inst. **11:** 343–356. *f. 1–5.* O–D 1940.

Tillson, A. H. The floral anatomy of the Kalanchoideae. Am. Jour. Bot. **27:** 595–600. *f. 1–30.* 12 N 1940.

Tinney, F. W. & Aamodt, O. S. The progeny test as a measure of the types of seed-development in *Poa pratensis* L. Jour. Hered. **31:** 457–464. *f. 18–22.* O 1940.

Vallega, J. Especialización fisiologica de *Puccinia graminis tritici* en la Argentina, Chile y Uruguay. Inst. Fito. Catalina Publ. **12:** 196–220. 1940.

Vestal, P. A. Wood anatomy as an aid to classification and phylogeny. Chron. Bot. **6:** 53, 54. N 1940.

Viégas, A. P. Anatomia da parte vegetativa da *Mandioca.* Inst. Agron. São Paulo Bol. Tec. **74:** 1–20. *f. 1–65.* Je 1940.

Viegas, A. P. Notas sôbre *Cyphella villosa* Pers. Inst. Agron Sao Paulo Bol. Tec. **80:** 1–7. *f. 1–9* 1940.

Viegas, A. P. Notas sôbre *Septobasidium pseudopedicellatum* Burt o causador dum dos filtros dos feltros dos citrus no estado de sao Paulo. Inst. Agron. Sao Paulo Bol. Tec. **79:** 1–6. *f. 1–6.* 1940.

Viégas, A. P. Observações acerca de uma *Auricularia* comum no estado São Paulo. Rodriguesia **4:** 279–281. *5 pl.* 1940.

Viégas, A. P. Uma nova espécie do genero *Dendrothele.* Rodriguesia **4:** 283–287. *3 pl.* 1940.

Viegas, A. P. Uma variedade de *Septobasidium castaneum* Burt. Inst. Agron. Sao Paulo Bol. Tec. **73:** 1–7. *f. 1–8.* 1940.

Voth, P. D. & Hamner, K. C. Responses of *Marchantia polymorpha* to nutrient supply and photoperiod. Bot. Gaz. **102:** 168–205. *f. 1–14.* 15 O 1940.

Walker, E. G. Some problems and methods in the taxonomy of Chinese plants. Lingnan Sci. Jour. **19:** 583–594. *f. 1–4.* 26 O 1940.

Waterfall, U. T. Interesting plants of Oklahoma. Rhodora **42:** 499–502. N 1940.

Webber, J. M. Polyembryony. Bot. Rev. **6:** 575–598. N 1940.

Wehmeyer, L. E. Contributions to a study of the fungus flora of Nova Scotia. V. Discomycetes. Canad. Jour. Sci. **18:** 535–549. N 1940.

White, E. Principles of genetics. 1–352. *f. 1–179.* St. Louis, Mosby, 1940.

Williams, L. O. A new *Epidendrum* from Mexico. Bull. Am. Orchid Soc. **9:** 97. *1 f.* S 1940.

Williams, L. O. A new *Mormodes* from Mexico. Bull. Am. Orchid Soc. **9:** 153–155. *illust.* 1 N 1940.

Williams, L. O. A new species of *Bulbophyllum* from Mexico. Bull. Am. Orchid Soc. **9**: 21. *pl. 20*. 1940.

Williams, L. O. A new species of *Pleurothallis* from Mexico. Bull. Am. Orchid Soc. **9**: 69. *pl. 68*. Au 1940.

Williams, L. O. Two new species of *Hexalectris* from Mexico. Bull. Am. Orchid Soc. **9**: 125, 126. *1 f*. O 1940.

Wolf, F. T. Macrosporogenesis and the development of the embryo sac in *Yucca aloifolia*. Bull. Torrey Club **67**: 755–761. *f. 1–15*. 3 D 1940.